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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

001560-350

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)

Unassigned **09/147955**INTERNATIONAL APPLICATION NO.  
PCT/JP98/03199INTERNATIONAL FILING DATE  
16 July 1998PRIORITY DATE CLAIMED  
25 July 1997

TITLE OF INVENTION

GENE CODING FOR A PROTEIN HAVING GLYCOSIDE TRANSFER ACTIVITY

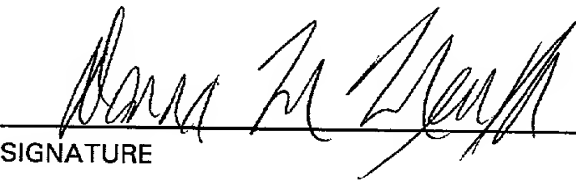
APPLICANT(S) FOR DO/EO/US

Masako MIZUTANI, Yoshikazu TANAKA, Takaaki KUSUMI, Kazuki SAITO, Mami YAMAZAKI, and Gong ZHIZHONG

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
  3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
  4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
  5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☒ has been transmitted by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
  7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
    - b. ☐ have been transmitted by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☒ have not been made and will not be made.
  8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
  9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
  10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11. to 16. below concern other document(s) or information included:**
11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☒ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
  14. ☐ A substitute specification.
  15. ☐ A change of power of attorney and/or address letter.
  16. ☒ Other items or information:

Copy of International Search Report, and a copy of Notice Informing Applicant of the Communication of the International Application to the Designated Offices.

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50) <b>Unassigned</b>		INTERNATIONAL APPLICATION NO. <b>PCT/JP 98/03199</b>		ATTORNEY'S DOCKET NUMBER <b>001560-350</b>	
17. <input checked="" type="checkbox"/> The following fees are submitted:				<b>CALCULATIONS</b>	PTO USE ONLY
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b>  Search Report has been prepared by the EPO or JPO ..... \$840.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$760.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$970.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$96.00  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>					
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30					
Claims	Number Filed	Number Extra	Rate		
Total Claims	19 -20 =	0	X\$18.00		
Independent Claims	1 -3 =	0	X\$78.00		
Multiple dependent claim(s) (if applicable)			+ \$260.00		
<b>TOTAL OF ABOVE CALCULATIONS =</b>					
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					
<b>SUBTOTAL =</b>					
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30 +					
<b>TOTAL NATIONAL FEE =</b>					
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +					
<b>TOTAL FEES ENCLOSED =</b>					
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>880.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:  Ronald L. Grudziński, Esq. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404					
				 SIGNATURE	
				Donna M. Meuth NAME	
				<u>36,607</u> REGISTRATION NUMBER	

09/147955  
510 Rec'd PCT/PTO 24 MAR 1999

Patent  
Attorney's Docket No. 001560-350

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
 )  
Masako MIZUTANI et al ) Group Art Unit: Unassigned  
 )  
Application No.: Unassigned ) Examiner: Unassigned  
Corresponding to PCT/JP 98/03199 )  
 )  
Filed: March 24, 1999 )  
 )  
For: GENE CODING FOR A PROTEIN )  
HAVING GLYCOSIDE TRANSFER )  
ACTIVITY )

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above identified  
application as follows:

**IN THE SPECIFICATION:**

In compliance with 37 C.F.R. § 1.823(a), please substitute the attached copy  
of the "Sequence Listing" for the current "Sequence Listing" at pages 22-39 of the above-  
identified application.

**IN THE CLAIMS:**

Please amend claims 6, 8 and 10 as follows:

In claim 6, lines 1 and 2, please delete "any one of claims 1 through 5" and insert therefore --claim 1--.

In claim 8, lines 1 and 2, please delete "any one of claims 1 through 5" and insert therefore --claim 1--.

In claim 10, line 2, please delete "any one of claims 1 through 5" and insert therefore --claim 1--.

Please insert the following new claims 12-19 as follows:

--12. A protein encoded by a gene as set forth in claim 2.

13. A protein encoded by a gene as set forth in claim 3.

14. A protein encoded by a gene as set forth in claim 4.

15. A protein encoded by a gene as set forth in claim 5.

16. A plant into which is introduced a gene as set forth in claim 2, or its progeny or tissue having identical properties.

17. A plant into which is introduced a gene as set forth in claim 3, or its progeny or tissue having identical properties.

18. A plant into which is introduced a gene as set forth in claim 4, or its progeny or tissue having identical properties.

19. A plant into which is introduced a gene as set forth in claim 5, or its progeny or tissue having identical properties.--

**REMARKS**

Entry of the foregoing and examination of the above-identified application is respectfully requested.

The paper copy of the Sequence Listing for the subject application, is by this amendment, substituted for the current Sequence Listing at pages 22-39 and before the claims of the above-identified application. Please renumber the pages accordingly.

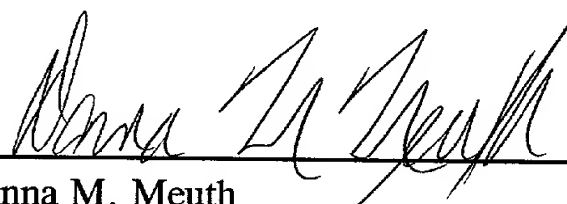
Claims 6, 8 and 10 have been amended to eliminate the multiple dependency of the claims and to place them in better form in accordance with U.S. practice. New claims 12-19 have been added directed to preferred embodiments. Support for these claims may be found at the very least in original claims 8 and 10.

Early and favorable action in the form of Notice of Allowance is respectfully requested.

In the event that there are any questions relating to this amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone so that prosecution would be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By:   
Donna M. Meuth  
Registration No. 36,607

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Date: March 24, 1999

GENE CODING FOR A PROTEIN HAVING GLYCOSIDE TRANSFER  
ACTIVITY

5 Technical Field

The present invention relates to a gene coding for a protein having activity that transfers a glycoside to the 5 position of a flavonoid, and a process utilizing that gene.

10

Background Art

The flower industry strives to develop various new varieties. Changing the color of a flower is one way of effectively breeding a new variety. A wide range of colors have been successfully produced for nearly all commercial varieties using classical breeding methods. With these methods, however, since there are restrictions on the gene pool for each species, it is rare for a single species to have a broad range of colored varieties.

20 Flower colors are based on two types of pigments, namely flavonoids and carotinoids. Flavonoids contribute to color tones ranging from yellow to red and blue, while carotinoids contribute to color tones of orange or yellow. Flavonoid molecules that primarily contribute to flower color are anthocyanins which are glycosides of cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin, and different anthocyanins cause remarkable changes in flower color. Moreover, flower color is also affected by auxiliary coloring by colorless flavonoids, metal complex formation, glucosylation, acylation, methylation and vacuolar pH (Forkmann, Plant Breeding, 106, 1, 1991).

25 The biosynthesis route of anthocyanins, which begins with phenylalanine, has been well understood (e.g., Plant Cell, 7, 1071-1083, 1995), and nearly all genes involved in the biosynthesis have been cloned. For example, among those genes thought to be involved in biosynthesis of

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malonylshisonin (3-0-(6-0-(p-cumaloyl)- $\beta$ -D-glucosyl)-5-0-(6-0-malonyl- $\beta$ -D-glucosyl)-cyanidin), which is an anthocyanin of Perilla, those genes for which homologues have not yet been reported are only the flavonoid-3'-hydroxylase, UDP-glucose:anthocyanin (flavonoid) 5-0-glucosyl transferase (abbreviated as 5GT) and malonyl group transferase genes.

Among these, flavonoid-3'-hydroxylase is known to belong to the cytochrome P450 gene family (Plant Cell, 7, 1071-1083, 1995), and cytochrome P450 genes are surmised to demonstrate structural homology.

The hydroxyl group at the 3 position of flavonoid molecules is typically modified by glucose, and generally glucosylation and other modifications by glycoside are considered to increase the stability and solubility of anthocyanins (The Flavonoids, Chapman & Hall, 1994).

Genes coding for the UDP-glucose:anthocyanidin or flavonoid-3-glucosyl transferase (abbreviated as 3GT) that catalyze this reaction are obtained from numerous plants such as corn, barley, snapdragons and gentians, and their amino acid sequences mutually demonstrate significant homology. For example, the homology between the 3GT amino acid sequences of monocotyledonous corn and dicotyledonous gentian is 32%, that between the 3GT amino acid sequences of monocotyledonous corn and monocotyledonous barley is 73%, and that between the 3GT amino acid sequences of dicotyledonous gentian and dicotyledonous eggplant is 46%.

In addition, the gene coding for UDP-ramnose:anthocyanidin 3-glucosidoramnosyl transferase (3RT) of petunias has also been cloned.

However, even though the hydroxyl group at the 5 position of the flavonoids of numerous plants is glucosylated, a gene for the enzyme (5GT) that catalyzes this reaction has yet to be obtained.

In addition, although there are examples of measuring the reaction by which glycoside is transferred to the 5



position of petunia and stock anthocyanins (Planta, 160, 341-347, 1984, Planta, 168, 586-591, 1986), these reports only describe the investigation of enzymological properties using crude extracts or partially purified products of flower petals, and there are no examples of this enzyme being purified to its pure form. In addition, since glycosyltransferases are typically biochemically unstable, enzyme purification is difficult.

Although there are hardly any cases in which color tone is changed by addition of glycoside to a flavonoid molecule, since aromatic acyl groups that have a significant effect on color tone are linked to a glucose molecule or rhamnose molecule within an anthocyanin, regulation of the glycoside transfer reaction is important in terms of controlling anthocyanin biosynthesis, and ultimately in controlling flower color. Furthermore, as an example of changing flower color by regulating the expression of glycosyltransferase gene, the reaction by petunia 3RT has been controlled in transformed petunia to modify flower color.

Plant species, which can be transformed with a foreign gene, include, for example, roses, chrysanthemums, carnations, daisies, petunias, torenia, bellflowers, calanchoes, tulips and gladiolas.

#### Disclosure of the Invention

The inventors of the present invention therefore sought to obtain a gene that codes for a protein having activity that transfers a glycoside to the 5 position of a flavonoid, thereby leading to completion of the present invention.

For example, the 5 position hydroxyl group of the anthocyanins of chrysanthemums and some of the anthocyanins of roses and carnations are not glucosylated. The anthocyanin structure can be changed by introducing the 5GT gene obtained by the present invention into these plants.

In addition, although it is possible to change flower color and stabilize flavonoids by acylating flavonoids using the acyl group transferase gene described in International Publication No. WO96/25500, since the acyl group does not bond directly with the flavonoid, but rather bonds by way of a sugar, simply introducing an acyl group transferase gene alone is not sufficient for changing flower color and may even cause the flavonoid not to become stable.

However, by introducing the 5GT gene in combination with an acyl group transferase gene, sugar is bounded to the 5 position of the flavonoid thereby further allowing the flavonoid to be acylated. This can be expected to change the anthocyanin structure and cause the flower color to become bluish.

In addition, if expression of 5GT gene of a plant in which the 5 position of anthocyanin is glucosylated is suppressed with the antisense method or co-suppression method and so forth, transfer of glucose residue to 5 position can be inhibited. So that, flower color can be changed. For example, suppressing 5GT activity in gentian or bellflower can be expected to cause flower color to become reddish.

The inventors of the present invention isolated cDNA of 5GT from Perilla, torenia, verbena and petunia plants using gene recombination technology, and determined the nucleotide sequence of the structural gene. Namely, the inventors of the present invention provide a DNA sequence that codes for 5GT present in the tissue that expresses anthocyanins in these plants. Moreover, since this enzyme transfers glycoside to the 5 position of anthocyanin pigment, it can be used to change flower color and increase anthocyanin stability.

#### Embodiment for Carrying Out the Invention

The method of differential displacement, for example, can be used to obtain DNA that codes for the enzyme of the

present invention. In *Perilla* (*Perilla frutescens*), for example, there are varieties that accumulate anthocyanins (e.g., red forma) and those that do not (e.g., green forma). By cloning DNA present in varieties that  
5 accumulate anthocyanins but not present in varieties that do not, it is possible to obtain the DNA that codes for the enzyme of the present invention.

More specifically, RNA is extracted from the leaves of red forma and green forma, and cDNA is synthesized in  
10 accordance with standard methods. This is then separated by electrophoresis to isolate cDNA present in the cDNA library of red forma but not present in the cDNA library of green forma. Next, the red forma cDNA library is screened using the resulting cDNA as a probe to obtain the  
15 cDNA that codes for the enzyme of the present invention.

Once cDNA that codes for the enzyme of the present invention is obtained in the manner described above, this cDNA or its fragment is used as a probe to screening the cDNA libraries of other plants. As a result, the DNA that  
20 codes for the enzyme of the present invention can be obtained from those plants.

As an example of the screening, in the present invention, the DNA coding for the enzyme of the present invention is cloned from *Perilla* by the differential  
25 display method (Example 1). Next, DNA that codes for the enzyme of the present invention is obtained from verbena by screening of cDNAs from verbena (*Verbena hybrida*) using the cloned DNA of Example 1 as a probe (Example 2). Moreover, DNA coding for the enzyme of the present  
30 invention is obtained from torenia in the same manner (Example 3).

Then, it was confirmed that the proteins encoded in these DNAs have the enzymatic activity of the present invention.

35 Moreover, the DNA coding for the enzyme of the present invention was obtained from petunia (Example 4).

Examples of the DNAs of the present invention include

that which codes for the amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12. However, proteins having an amino acid sequence modified by addition and/or deletion of one or more amino acids and/or substitutions by one or more other amino acids are also known to maintain enzymatic activity similar to the original protein. Thus, genes coding for a protein that has an amino acid sequence modified by addition and/or deletions of one or more amino acids and/or substitutions by one or more other amino acids relative to the amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and still maintains activity of transferring a glycoside to the 5 position of a flavonoid, also belong to the present invention.

The present invention also relates to a gene coding for a protein which gene hybridizes to a nucleotide sequence described in any one of SEQ ID NOs: 1 through 4 or 6, or to a nucleotide sequence that codes for an amino acid sequence described therein or to their portions, for example a portion coding for at least six amino acids of a consensus region, under conditions of 2 to 5 x SSC, and for example, 5 x SSC, and 50°C, and that has activity of transferring a glycoside to the 5 position of a flavonoid. Furthermore, the optimum hybridization temperature varies according to the nucleotide sequence and its length, and it is preferable that the hybridization temperature be lower the shorter the nucleotide sequence. For example, a temperature of 50°C or lower is preferable in the case of a nucleotide sequence (18 bases) coding for six amino acids.

Although examples of genes selected by hybridization in this manner include those which are naturally-occurring such as those derived from plants, examples of which include a gene derived from verbena and torenia, they may also be those derived from other plants, examples of which include petunias, roses, carnations and hyacinths. In addition, genes selected by hybridization may also be cDNA or genomic DNA.

Moreover, the present invention also relates to a gene coding for a protein having an amino acid sequence having homology of 30% or more, preferably 50% or more, for example 60% or 70% or more, and in some cases, 90% or more relative to an amino acid sequence of any of SEQ ID NOs: 7 through 10 or 12, and having activity that transfers a glycoside to the 5 position of a flavonoid. Namely, as indicated in Examples, DNA coding for the enzyme of the present invention demonstrates homology of 20 to 30% in comparison with other glycosyltransferase genes. Thus, the present invention includes genes coding for a protein that having homology of 30% or more with an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and has glycosyltransferase activity.

In addition, as is clear from a comparison of the results of Examples 1 through 4, the amino acid sequence of the enzyme of the present invention varies according to the species, with interspecies homology being 50% or more (see Examples 3 and 4), and for example 60 to 70% (see Example 2), while the homology of the amino acid sequences of the enzymes derived from the same species is 90% or more (see Example 1). Thus, genes coding for a protein that has an amino acid sequence having homology of 50% or more, for example 60-70% or more, and in some cases, 90% or more, relative to an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and maintains the glycosyltransferase activity of the present invention are included in the present invention.

As is described in detail in Examples, DNA having a native nucleotide sequence is obtained by, for example, screening of a cDNA library.

In addition, DNA coding for an enzyme having a modified amino acid sequence can be synthesized using ordinary site-specific mutagenesis and PCR based on the nucleotide sequence of a native DNA. For example, a DNA fragment containing a site at which a modification is desired to be introduced is obtained by restriction enzyme

digestion of cDNA or genomic DNA obtained as described above. By using this as a template, site-specific mutagenesis or PCR is performed using a primer containing the desired mutation to obtain a DNA fragment containing the desired modification. This is then ligated to DNA coding for another portion of the target enzyme.

Alternatively, in order to obtain DNA coding for an enzyme having a shortened amino acid sequence, for example, DNA coding for an amino acid sequence that is longer than the target amino acid sequence, for example that coding for the entire amino acid sequence, is digested by a desired restriction enzyme, and in the case the resulting DNA fragment does not code for the entire target amino acid sequence, the deficient portion should be supplemented by ligating synthetic DNA.

In addition, by expressing this clone using a gene expression system in E. coli or yeast and measuring enzyme activity, the resulting gene can be confirmed to code for glycosyltransferase, and by clarifying the translation region of glycosyltransferase gene that transfers glycoside to the 5 position of a flavonoid, a gene is obtained that codes for the glycosyltransferase claimed in the present invention. Moreover, by expressing said gene, the target transferase protein that transfers a glycoside to the 5 position of a flavonoid can be obtained.

Alternatively, the protein can be obtained by using antibody to an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12.

Thus, the present invention also relates to a recombinant vector containing the above-mentioned DNA, and more particularly, to an expression vector and a host transformed with the vector. Both prokaryotes and eukaryotes can be used for the host. Examples of prokaryotes that can be routinely used for the host include bacteria, for example, the genus Escherichia such as Escherichia coli, and the genus Bacillus such as Bacillus subtilis.

Examples of eukaryotes that can be used include lower eukaryotes such as eucaryotic microorganisms including fungi such as yeast or mold. Examples of yeast includes the genus Saccharomyces such as Saccharomyces cerevisiae,  
5 while examples of molds include the genus Aspergillus such as Aspergillus oryzae and Aspergillus niger, as well as the genus Penicillium. Moreover, animal or plant cells can also be used, examples of animal cells including mouse, hamster, monkey and human cell systems. Moreover,  
10 insect cells such as silkworm cells or adult silkworms themselves can be used as hosts.

The expression vectors of the present invention contain an expression control region, such as a promoter, terminator or an origin of replication, depending on the  
15 type of host in which they are to be introduced. Examples of promoters of bacterial expression vectors include conventionally used promoters such as trc promoter, tac promoter and lac promoter, while examples of yeast promoters include glyceroaldehyde triphosphate  
20 dehydrogenase promoter and PH05 promoter. Examples of mold promoters include amylase and trpC. In addition, examples of promoters for animal cell hosts include viral promoters such as SV40 early promoter and SV40 late promoter.

25 Preparation of expression vector can be performed in accordance with standard methods using restriction enzyme, ligase and so forth. In addition, transformation of a host by an expression vector can also be performed in accordance with standard methods.

30 In the process for producing the above-mentioned protein, a host transformed with the expression vector is cultured, cultivated or bred, the target protein can be recovered and purified from the resulting culture in accordance with standard methods, examples of which  
35 include filtration, centrifugation, cell homogenation, gel filtration chromatography and ion exchange chromatography.

Furthermore, although the present specification

describes transferases derived from Perilla, verbena,  
torenia and petunia wherein the transferases that transfer  
glycoside to the 5 position of a flavonoid (which may be  
simply referred to as "glycosyltransferase" in the present  
invention), a gene that codes for said enzyme can be  
cloned, by entirely or partially altering the purification  
method of said enzyme so as to purify a  
glycosyltransferase of another plant, and determining the  
amino acid sequence of said enzyme. Moreover, by using  
cDNA of the glycosyltransferase derived from Perilla of  
the present invention as a probe, cDNA of a different  
glycosyltransferase was able to be obtained from Perilla,  
and cDNA of a different glycosyltransferase was able to be  
obtained from a different plant. Thus, other  
glycosyltransferase genes can be obtained by using a  
portion or the entirety of a glycosyltransferase gene.

In addition, as indicated in the present  
specification, by purifying glycosyltransferase from  
Perilla, verbena, torenia and petunia to obtain antibody  
to said enzyme in accordance with standard methods, cDNA  
or chromosomal DNA produces protein which reacts with that  
antibody that can be cloned. Thus, the present invention  
is not limited to only genes of glycosyltransferases  
derived from Perilla, verbena, torenia and petunia, but  
also relates to glycosyltransferase in the broad sense.

Moreover, the present invention also relates to a  
plant, its progeny or their tissue for which color has  
been adjusted by introduction of glycosyltransferase gene,  
and their form may be that of cut flowers as well.

In addition, UDP-glucose is an example of a glycoside  
donor in the glycoside transfer reaction of glycoside that  
include anthocyanin in the present specification.

#### Examples

The following provides a detailed explanation of the  
present invention based on Examples. Unless specified  
otherwise, the experimental procedure was performed in



accordance with the methods described in Molecular Cloning (Cold Spring Harbor, 1989), New Biochemistry Experimental Manual (Kagaku Dojin, 1996) and International Patent Laid-Open Publication No. WO96/25500.

5        Example 1 Cloning of a Gene Specifically Expressed in  
         Red Forma

(1) Differential Display

Perilla (Perilla frutescens) includes varieties that accumulate anthocyanins in their leaves (for example, red  
10 forma (Sakata-no-tane)), and varieties that do not accumulate anthocyanins (for example, blue forma (Sakata-no-tane)). The structure of the major anthocyanin is reported to be malonylshisonin (3-0-(6-0-(p-cumaloyl)- $\beta$ -D-glucosyl)-5-0-(6-0-malonyl- $\beta$ -D-glucosyl)-cyanidin) (Agri.  
15 Biol. Chem., 53:197-198, 1989).

Differential display is a method reported in Science, 257, 967-971 (1992), and is used, for example, to obtain genes that are expressed tissue-specifically.

Total RNA was extracted from the leaves of the above-mentioned two types of Perilla by the hot phenol method  
20 (Plant Molecular Biology Manual, Kluwer Academic Publishers, 1994, pp. D5/1-13). Poly A + RNA was purified from the resulting total RNA using an mRNA separator kit (Clonetech). 0.9  $\mu$ g of poly A + RNA were reverse-  
25 transcribed in 33  $\mu$ l of reaction mixture using oligo-dT primer added an anchor (GenHunter, H-T11G, H-T11A and H-T11C) to obtain single strand cDNA. Using this cDNA as a template, PCR was performed using the same oligo-dT primer added an anchor and synthetic primers (GenHunter, H-AP1  
30 through 8) as primers.

The volume of the PCR reaction mixture was 20  $\mu$ l, and it contained 2  $\mu$ l of cDNA solution, 0.2  $\mu$ M of any one of H-T11G, H-T11A or H-T11C primer, 0.2  $\mu$ M of any primer from H-AP1 through H-AP8, 0.12  $\mu$ M dNTP, 5 or 10  $\mu$ Ci of  
35 [<sup>32</sup>P]dCTP, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.01% Triton X-100, 1.25 mM MgCl<sub>2</sub> and 1 unit of Taq polymerase. The reaction conditions comprised holding the temperature at

72°C for 20 seconds followed by repeating the reaction for 40 cycles with one cycle comprising raising the temperature to 94°C for 30 seconds, lowering to 40°C for 2 minutes and raising to 72°C for 30 seconds, and then  
5 holding the temperature at 72°C for 5 minutes.

The DNA fragments amplified in this manner were separated by the same polyacrylamide gel electrophoresis as used for DNA Sequencing. After drying the gel, the gel was exposed to X-ray film. Among the resulting  
10 approximately 2,600 bands, there were 36 bands observed only in the red forma as a result of comparing the two varieties. They were cut out of the dried gel and eluted into 100 µl of water. The eluted DNA was precipitated with ethanol and dissolved in 20 µl of water. Using a  
15 half amount of each DNA as a template, the PCR reaction was performed as described above, and amplified fragments were obtained for 33 of DNA fragments. Library screening and northern analysis were then performed using these DNA fragments.

## 20 (2) Northern Analysis

Northern analysis was performed according to the method described below using the above 33 types of DNA probes. After separating poly A + RNA derived from red forma and green forma with formamide gel containing 1.2%  
25 agarose, the poly A + RNA was transferred to a Nylon membrane. This membrane was hybridized with the above-mentioned DNA probes labeled with [<sup>32</sup>P] for overnight at 65°C in the presence of 5XSSPE, 5X Denhalt's solution, 0.5% SDS and 20 µg/ml of denatured salmon sperm DNA. The  
30 hybridized membrane was washed at 65°C in 1XSSPE and 0.1% SDS solution and subjected to autoradiography. As a result, only five probes were specifically expressed in red forma. These clones are predicted to be genes involved in the biosynthesis of anthocyanins.

## 35 (3) Screening of cDNA Library

A cDNA library with λgt10 as a vector was prepared using the poly A + RNA obtained from the leaves of red

forma and the Complete Rapid Cloning System  $\lambda$ gt10 (Amersham). This cDNA library was screened with the five DNA fragments described above to obtain cDNA corresponding to each fragment. Among these, a clone named 3R5 was  
5 obtained using a DNA fragment obtained by H-T11A and H-AP3 primers, and this clone demonstrated homology of approximately 26% at the amino acid level with previously reported corn flavonoid-3-O-glucosyl transferase.

In addition, clones designated as 3R4 and 3R6 were  
10 obtained by library screening using the same probes, and these demonstrated an extremely high level of homology with 3R5. The complete nucleotide sequences and deduced amino acid sequences of 3R4 and 3R6 are shown in SEQ ID NO: 1 and SEQ ID NO: 2 of the Sequence Listing,  
15 respectively. In addition, the deduced amino acid sequences of the proteins encoded by 3R4 and 3R6 demonstrated homology of 92%.

A clone designated as 8R6 was obtained using a DNA fragment obtained by H-T11G and H-AP8 primers, and this  
20 clone did not demonstrate significant homology with any sequences reported so far. This sequence is shown in SEQ ID NO: 5 of the Sequence Listing. Although there is a strong possibility that 8R6 is a gene involved in the biosynthesis of anthocyanins, since its structure lacks  
25 homology with genes reported so far, it is predicted to be a new gene involved in anthocyanin biosynthesis.

In consideration of the anthocyanin structure in Perilla (the previously mentioned malonylshisonin), it is predicted that this gene is a malonyl transferase. In  
30 order to verify this, this gene should be expressed in yeast and *E. coli* followed by reacting with anthocyanin and malonyl-CoA as substrates. Such an experiment can be carried out using, for example, the method described in International Publication No. WO96/25500. Malonyl  
35 transferase gene is useful in terms of artificially altering anthocyanin structure.

(4) Expression of 3R4 cDNA in Yeast

An approximately 1.5 kb DNA fragment obtained by blunting the BstXI cleaved site of p3R4 using T4 DNA polymerase (Takara Shuzo) and then cutting out at the BamHI cleavage site in the adapter, and an approximately 8 kb DNA fragment obtained by blunting the EcoRI cleaved end of pYE22m and then digesting with BamHI were ligated to obtain a plasmid that was designated as pY3R4.

Furthermore, E. coli strain JM109 having pYE22m was named Escherichia coli SBM335, and deposited at the National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology as FERM BP-5435. In pY3R4, cDNA coding for glycosyltransferase has been ligated downstream of the promoter for glyceroaldehyde triphosphate dehydrogenase lone of the constitutive yeast promoter, and transcription is controlled by this promoter.

Using pY3R4, yeast Saccharomyces cerevisiae G1315 (Ashikari, et al., Appl. Microbiol. Biotechnol., 30, 515-520, 1989) was transformed according to the method of Ito, et al. (Ito, et al., J. Bacteriol., 153, 163-168, 1983). The transformed yeast was selected according to recovery of tryptophan synthesis ability. The resulting transformed strain was cultured for 24 hours at 30°C with shaking in 10 ml of Burkholder's medium (Burkholder, Amer. J. Bot., 30, 206-210) containing 1% casamino acids.

In order to conduct a control experiment, yeast that spontaneously recovered tryptophan synthesis ability was also cultured in the same manner. After collecting the yeast, the cells were suspended in suspension buffer (100 mM phosphate buffer (pH 8.5), 0.1% (v/v) 2-mercaptoethanol, 10  $\mu$ M APMSF and 100  $\mu$ M UDP-glucose) followed by the addition of glass beads (Glass Beads, 425-600 microns Acid-Wash, Sigma) and vigorous shaking to crush the cells. The crushed cells were then centrifuged for 20 minutes at 15,000 rpm and the supernatant was used as a crude enzyme solution for the measurement of enzyme activity described below.

(5) Measurement of Enzymatic Activity

After allowing 50  $\mu$ l of reaction mixture containing 20  $\mu$ l of crude enzyme solution (100 mM phosphate buffer (pH 8.5), 670  $\mu$ M cyanidin-3-glucoside, 1 mM UDP-glucose) for 10 minutes at 30 °C, 50  $\mu$ l of 50% acetonitrile solution containing 0.1% TFA was added to stop the reaction. Supernatant obtained by centrifuging for 5 minutes at 15,000 rpm was passed through a Samprep LCR4(T)-LC filter (Millipore) so as to remove impurities. This was then analyzed by high-performance liquid chromatography (HPLC). Analysis was performed using a reverse phase column (Asahipak ODP-50, 4.6 mm diameter x 250 mm, Showa Denko), the mobile phase consisted of 0.5% TFA/H<sub>2</sub>O for solution A and 0.5% TFA 50% CH<sub>3</sub>CN for solution B. The flow rate was 0.6 ml/min. and the fractions were eluted at a gradient of B20%  $\rightarrow$  B100% (20 min) followed by holding at B100% for 5 minutes.

20  $\mu$ l of reaction mixture was used for analysis. A520 nm, AUFS 0.5 (Shimadzu SPD-10A) and a photodiode array detector (Shimadzu SPD-M6A) at an absorbance of 600-250 nm were used for detection. In the case of reaction of yeast crude enzyme solution that expressed pY3R4, in addition to the substrate cyanidin-3-glucoside (retention time: 17 minutes), a new peak was observed at retention time of 14.5 minutes. Since it was not observed in the case of reaction of yeast crude enzyme solution of the control experiment, this new peak was considered to be generated due to the activity of protein originated from pY3R4. As a result of co-chromatography with cyanidin-3,5-diglucoside, the retention time of this peak coincided with that of cyanidin-3,5-diglucoside, and their absorption spectra were also identical to each other. Based on these observations, 3R4 cDNA of Perilla was found to code for 5GT.

Example 2 Cloning of 5GT Gene of Verbena hybrida

(1) Preparation of cDNA Library

Petals were collected from Verbena variety Hanatemari

094496009962460  
violet (Suntory) and ground by a mortar and pestle in  
liquid nitrogen. RNA was extracted from the ground  
tissues according to a method using guanidine  
thiocyanate/cesium chloride, and poly A + RNA was obtained  
5 by the method recommended by the manufacturer using  
Oligotex (Takara Shuzo). The method using guanidine  
thiocyanate/cesium chloride was carried out in accordance  
with the method described in detail in Methods in  
Molecular Biology, Vol. 2 (Humana Press Inc., 1984) by R.  
10 McGookin and Robert J. Slater, et al.

Using the resulting poly A + RNA as a template,  
double-stranded cDNA was synthesized using the ZAP-cDNA  
synthesis kit (Stratagene), then, a cDNA library was  
prepared using the Uni-ZAP XR Cloning Kit (Stratagene)  
15 according to the method recommended by the manufacturer.

#### (2) Cloning of 5GT cDNA

The  $\lambda$  phage library obtained as described above was  
screened in the following manner using the p3R4 cDNA of  
Perilla as a probe. The filters were maintained at 42°C  
20 for 1 hour in hybridization buffer (5X SSC, 30% formamide,  
50 mM sodium phosphate buffer (pH 7.0), 3% SDS 2% blocking  
reagent (Boehringer), 0.1% lauroylsarcosine, 80  $\mu$ g/ml of  
salmon sperm DNA). DIG-labeled Perilla 5GT cDNA, p3R4  
cDNA, fragment was added to the hybridization solution and  
25 the filters were incubated for further 16 hours.

After washing the filters with washing solution (5X  
SSC 50°C, 1% SDS), the positive clones labeled with anti-  
DIG-alkaline phosphate were immunologically detected using  
5-bromo-4-chloro-3-indolylphosphate and nitro blue  
30 tetrazolium salt according to the method described by the  
manufacturer (Boehringer).

As a result, seven positive clones were obtained.  
These cDNA were excised on plasmid pBluescript SK using  
the method recommended by Stratagene. When the lengths of  
35 the cDNA were investigated by agarose gel electrophoresis,  
insertion of a maximum length of 2.0 kb was observed.

#### (3) Determination of Nucleotide Sequence

Plasmids were extracted from the resulting clones, and the nucleotide sequences near the 3' and 5' ends of the cDNA were determined according to the dideoxy sequence method using fluorescent reagent as recommended by Perkin-Elmer with the ABI 373A sequencer (Perkin-Elmer). As a result, five of the seven clones had mutually same nucleotide sequences although the lengths of the cDNA were different. The entire nucleotide sequence of pSHGT8 was determined. Determination of nucleotide sequences was performed as described above by either using the Kilo-Sequence Deletion Kit (Takara Shuzo) to obtain a series of deleted cDNA clones, or by using an oligoprimers specific for the internal sequence of pSHGT8.

#### (4) Comparison of the Nucleotide Sequence and the Amino Acid Sequence

The cDNA inserted into pSHGT8 had the length of 2062 bp, and included an open reading frame (ORF) consisting of 1386 bp in length (including a stop codon). This sequence is shown in SEQ ID NO: 3. The amino acid sequence of this ORF had homology of 68% with the amino acid sequence of Perilla 5GT encoded by p3R4, and homology of 64% with that encoded by p3R6. In addition, it also had homology of 22 to 25% with the 3GTs of monocotyledonous and dicotyledonous plants, and homology of 21% with petunia 3RT.

#### (5) Expression in Yeast and Measurement of Enzymatic Activity

An approximately 2.0 kb DNA fragment obtained by digesting pSHGT8 with BamHI/XhoI, and an approximately 8 kb DNA fragment obtained by digesting pYE22m with BamHI/SalI were ligated, and the resulting plasmid was designated as pYHGT8. pYHGT8 was expressed in yeast cells in the same manner as Example 1, and the enzymatic activity of the protein encoded by pSHGT8 was measured. As a result, in the reaction mixture containing the crude enzyme solution of yeast transformed with pYHGT8, a product was obtained that coincided with cyanidin-3,5-

diglucoside in both retention time and absorption spectrum. Based on this observation, the pSHGT8 cDNA of Verbena was determined to code for 5GT.

### Example 3 Cloning of Torenia 5GT Gene

#### 5 (1) Preparation of cDNA Library

Petals were collected from torenia variety Summer Wave Blue (Suntory) and ground in a mortar and pestle in liquid nitrogen. RNA was extracted from the ground tissues according to a method using guanidine thiocyanate/cesium chloride, and poly A + RNA was obtained by the method recommended by the manufacturer using Oligotex (Takara Shuzo). The method using guanidine thiocyanate/cesium chloride was carried out in accordance with the method described in detail in Methods in Molecular Biology, Vol. 10 2 (Humana Press Inc., 1984) by R. McGookin and Robert J. Slater, et al. 15

Using the resulting poly A + RNA as a template, double-strand cDNA was synthesized using the ZAP-cDNA synthesis kit of Strategene, then, a cDNA library was prepared using the Uni-ZAP XR Cloning Kit (Stratagene) according to the method recommended by the manufacturer. 20

#### (2) Cloning of 5GT cDNA

The  $\lambda$  phage library obtained as described above was screened in the same manner as Example 2 using the p3R4 cDNA of Perilla as a probe. As a result, eight positive clones were obtained. After excision of the cDNA on plasmid pBluescript SK, the lengths of the cDNA were investigated by agarose gel electrophoresis, which revealed that a maximum length of insertion was 1.6 kb. 25

#### 30 (3) Determination of Nucleotide Sequence

Plasmids were extracted from the resulting clones, and the nucleotide sequences near both 5' and 3' ends were determined in the same manner as Example 2. As a result, six of the eight clones were considered to have mutually same nucleotide sequences although the lengths of the cDNA were different. The entire nucleotide sequence of pSTGT5 cDNA was determined. 35



(4) Comparison of the Nucleotide Sequence and the Amino Acid Sequence

The cDNA encoded in pSTGT5 was of 1671 bp in length, and included an open reading frame (ORF) consisting of 1437 bp in length (including a stop codon). This sequence is shown in SEQ ID NO: 4. The amino acid sequence of this ORF had homology of 58% with the amino acid sequence of Perilla 5GT encoded by p3R4, and homology of 57% with that encoded by p3R6, and, homology of 57% with that encoded by Verbena pSHGT8. In addition, it also had homology of 19 to 23% with the 3GT of monocotyledonous and dicotyledonous plants, and homology of 20% with petunia 3RT.

(5) Expression of 5GT gene in Yeast

An approximately 1.6 kb DNA fragment obtained by digesting pSTGT5 with SmaI/KpnI, and an approximately 8 kb DNA fragment obtained by blunting the EcoRI-digested site of pYE22m and then digesting with KpnI were ligated, and the resulting plasmid was designated as pYTGT5. pYTGT5 was expressed in yeast cells in the same manner as Example 1, and the enzymatic activity of the protein encoded by pSTGT5 was measured. As a result, in the reaction mixture containing the crude enzyme solution of yeast transformed with pYTGT5, a product was obtained that coincided with cyanidin-3,5-diglucoside in both retention time and absorption spectrum. Based on this observation, the pSTGT5 cDNA of Torenia was determined to code for 5GT.

Example 4 Cloning of Petunia 5GT Gene

(1) Preparation of cDNA Library

A cDNA library was prepared by RNA extracted from petals of the Petunia variety Old Glory Blue in the manner described in detail by T. Holton, et al. (Plant Journal, 1993 4: 1003-1010)

(2) Cloning of 5GT cDNA

The cDNA library was screened in the same manner as Example 2 using the mixture of 5GT cDNAs of Perilla, torenia and verbena obtained in the manner described above

as probes. As a result, four positive cDNA clones were obtained and excised on plasmid pBluescript SK. The lengths of the cDNA were investigated by agarose gel electrophoresis, cDNA of a maximum length of 2.0 kb was observed.

### (3) Determination of the Nucleotide Sequence

Plasmids were extracted from the resulting clones, and the nucleotide sequence near the 5' end was determined in the same manner as Example 2. As a result, two of the four clones, pSPGT1, were appeared to code an amino acid sequence with a high degree of homology with those of 5GT from Perilla, torenia and verbena obtained thus far. Therefore, the entire nucleotide sequence of pSPGT1 was determined.

### (4) Comparison of the Nucleotide Sequence and the Amino Acid Sequence

The pSPGT1 cDNA was 2015 bp in length, and included an open reading frame (ORF) consisting of 1407 bp (including a stop codon). This sequence is shown in SEQ ID NO: 6. The amino acid sequence of this ORF had homology of 57% with that of 5GT encoded by p3R4 of Perilla, homology of 54% with that encoded by p3R6, 55% with that encoded by pSHGT8 of verbena, and 51% of that encoded by pTGT5 of torenia. In addition, it also had homology of 20 to 29% with the 3GT of monocotyledonous and dicotyledoneous plants, and homology of 20% with petunia 3RT. Based on this observation, pSPGT1 cDNA obtained from petunia is considered to code for 5GT.

### Industrial Applicability

As has been described above, cDNA coding for enzymes that transfer a glycoside to the 5 position of a flavonoid originating in Perilla, verbena, torenia and petunia were cloned and their nucleotide sequences were determined. In addition, the isolated cDNAs were clearly shown to code for 5GT by the enzymatic activity of their protein expressed in yeast. Introducing of these cDNAs into a

suitable plant expression vector and transferring the  
resulting expression constructs into a plant makes it  
possible to provide, increase or decrease 5GT activity in  
the transformed plant, which leads to regulation of flower  
5 color. In addition, by using this enzyme, the structure  
of anthocyanins can be altered or more stable anthocyanins can  
be synthesized either in plants or in vitro.

5GT activity in plants

CLAIMS

1. A gene coding for a protein having activity that transfers a glycoside to the 5 position of a flavonoid.

2. A gene as set forth in claim 1 that codes for a protein having an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12 and having activity that transfers a glycoside to the 5 position of a flavonoid, or a protein having an amino acid sequence modified by addition and/or deletion of one or more amino acids and/or substitutions by one or more other amino acids relative to said amino acids and maintains activity that transfers a glycoside to the 5 position of a flavonoid.

3. A gene as set forth in claim 1 that codes for a protein having an amino acid sequence that has homology of 30% or more with an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and has activity that transfers a glycoside to the 5 position of a flavonoid.

4. A gene as set forth in claim 1 that codes for a protein having an amino acid sequence that has homology of 50% or more with an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and has activity that transfers a glycoside to the 5 position of a flavonoid.

5. A gene as set forth in claim 1 that codes for a protein, wherein said gene can be hybridized under conditions of 5 x SCC and 50°C with all or a portion of a nucleotide sequence that codes for an amino acid sequence described in any one of SEQ ID NOs: 7 through 10 or 12, and has activity that transfers a glycoside to the 5 position of a flavonoid.

6. A vector containing a gene as set forth in any one of claims 1 through 5.

7. A host transformed with a vector as set forth in claim 6.

8. A protein encoded by a gene as set forth in any one of claims 1 through 5.

5

10.

11.

ABSTRACT

The present invention provides a gene that codes for a protein having an amino acid sequence described in any of  
5 SEQ ID NOs: 7 through 10 or 12 and having activity that transfers a glycoside to the 5 position of a flavonoid, a gene that codes for a protein having a modified amino acid sequence relative to the above amino acid sequence and having activity that transfers a glycoside to the 5  
10 position of a flavonoid, and a process for producing the above protein using said gene. This gene can be used to artificially alter the color of plants.

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Glu Ile Asp Ala Gly Ser Asp Ala Ile His Leu Pro Gly Gly Leu Pro	
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Val Leu Ala Gln Arg Asp Leu Pro Ser Phe Leu Leu Pro Ser Thr His	
170 175 180 185	
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Glu Arg Phe Arg Ser Leu Met Lys Glu Lys Leu Glu Thr Leu Glu Gly	
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Glu Glu Lys Pro Lys Val Leu Val Asn Ser Phe Asp Ala Leu Glu Pro	
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Asp Ala Leu Lys Ala Ile Asp Lys Tyr Glu Met Ile Ala Ile Gly Pro	
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Leu Ile Pro Ser Ala Phe Leu Asp Gly Lys Asp Pro Ser Asp Arg Ser	
235 240 245	
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Phe Gly Gly Asp Leu Phe Glu Lys Gly Ser Asn Asp Asp Asp Cys Leu	
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Glu Trp Leu Ser Thr Asn Pro Arg Ser Ser Val Val Tyr Val Ser Phe	
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Gly Ser Phe Val Asn Thr Thr Lys Ser Gln Met Glu Glu Ile Ala Arg	
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Gly Leu Leu Asp Cys Gly Arg Pro Phe Leu Trp Val Val Arg Val Asn	
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Glu Gly Glu Glu Val Leu Ile Ser Cys Met Glu Glu Leu Lys Arg Val	
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Gly Lys Ile Val Ser Trp Cys Ser Gln Leu Glu Val Leu Thr His Pro	
330 335 340 345	
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Ser Leu Gly Cys Phe Val Thr His Cys Gly Trp Asn Ser Thr Leu Glu	
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Ser Ile Ser Phe Gly Val Pro Met Val Ala Phe Pro Gln Trp Phe Asp	
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Gln Gly Thr Asn Ala Lys Leu Met Glu Asp Val Trp Arg Thr Gly Val	
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Arg Val Arg Ala Asn Glu Glu Gly Ser Val Val Asp Gly Asp Glu Ile	
395 400 405	
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Arg Arg Cys Ile Glu Glu Val Met Asp Gly Gly Glu Lys Ser Arg Lys	
410 415 420 425	
ctt aga gag agt gct ggc aag tgg aag gat ttg gca aga aaa gct atg	1348
Leu Arg Glu Ser Ala Gly Lys Trp Lys Asp Leu Ala Arg Lys Ala Met	
430 435 440	
gag gaa gat gga tct tca gtt aac aac ctc aag gtc ttt ctt gat gag	1396
Glu Glu Asp Gly Ser Ser Val Asn Asn Leu Lys Val Phe Leu Asp Glu	
445 450 455	

gtt gta ggt atc taaagacgta aatgaggtcc ccataggcaa aattgcaa at 1448

Val Val Gly Ile

460 461

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gactaacttt gtacaaaatg aaaagttata tgatgaaatt ttaaaaaaca aactcagaca 1868

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Met Val Asn

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Lys Arg His Ile Leu Leu Ala Thr Phe Pro Ala Gln Gly His Ile Asn

5

10

15

cct tct ctc gag ttc gcc aaa agg ctc ctc aac acc gga tac gtc gac 149

Pro Ser Leu Glu Phe Ala Lys Arg Leu Leu Asn Thr Gly Tyr Val Asp

20

25

30

35

caa gtc aca ttc ttc acg agt gta tac gca ttg aga cgc atg cgc ttc 197

Gln Val Thr Phe Phe Thr Ser Val Tyr Ala Leu Arg Arg Met Arg Phe

40

45

50

gaa acc gat ccg agc agc aga atc gat ttc gtg gca tkt yca gat tct 245

Glu Thr Asp Pro Ser Ser Arg Ile Asp Phe Val Ala Xaa Xaa Asp Ser

55

60

65

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Tyr Asp Asp Gly Leu Lys Lys Gly Asp Asp Gly Lys Asn Tyr Met Ser	
70 75 80	
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Glu Met Arg Lys Arg Gly Thr Lys Ala Leu Lys Asp Thr Leu Ile Lys	
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Leu Asn Asp Ala Ala Met Gly Ser Glu Cys Tyr Asn Arg Val Ser Phe	
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Val Val Tyr Ser His Leu Phe Ser Trp Ala Ala Glu Val Ala Arg Glu	
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Val Asp Val Pro Ser Ala Leu Leu Trp Ile Glu Pro Ala Thr Val Phe	
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Asp Val Tyr Tyr Phe Tyr Phe Asn Gly Tyr Ala Asp Asp Ile Asp Ala	
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Gly Ser Asp Gln Ile Gln Leu Pro Asn Leu Pro Gln Leu Ser Lys Gln	
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Asp Leu Pro Ser Phe Leu Leu Pro Ser Ser Pro Ala Arg Phe Arg Thr	
180 185 190 195	
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Leu Met Lys Glu Lys Phe Asp Thr Leu Asp Lys Glu Pro Lys Ala Lys	
200 205 210	
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Val Leu Ile Asn Thr Phe Asp Ala Leu Glu Thr Glu Gln Leu Lys Ala	
215 220 225	
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Ile Asp Arg Tyr Glu Leu Ile Ser Ile Gly Pro Leu Ile Pro Ser Ser	
230 235 240	
ata ttc tca gat ggc aac gac ccc tca tca agc aac aaa tcc tac ggt	821
Ile Phe Ser Asp Gly Asn Asp Pro Ser Ser Ser Asn Lys Ser Tyr Gly	
245 250 255	





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 455 460 465  
 ttt att act agg att att aat gaa aat gcc tca taagttgtac 1488  
 Phe Ile Thr Arg Ile Ile Asn Glu Asn Ala Ser  
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 Met Ser  
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 Ser Ser Ser Ser Arg Arg Trp Arg Glu Asn Glu Gly Met Arg Arg Thr  
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 Leu Leu Gly Leu Gly Leu Gly Gln Leu Val Ser Phe Asp Leu Ala Ile  
 20 25 30  
 atg acc ttt tct gct tct ttg gtt tca acc aca gtg gat gca cca ctt 443  
 Met Thr Phe Ser Ala Ser Leu Val Ser Thr Thr Val Asp Ala Pro Leu  
 35 40 45 50  
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 Thr Met Ser Phe Thr Thr Tyr Thr Val Val Ala Leu Leu Tyr Gly Thr  
 55 60 65  
 atc ttg ctt tac cgc cgc cac aaa ttc ttg gtt cca tgg tac tgg tat 539  
 Ile Leu Leu Tyr Arg Arg His Lys Phe Leu Val Pro Trp Tyr Trp Tyr  
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Ala Leu Leu Gly Phe Val Asp Val His Gly Asn Tyr Leu Val Asn Lys	
85 90 95	
gca ttc gag ttg aca tcg att acg agt gtg agc ata ctg gat tgt tgg	635
Ala Phe Glu Leu Thr Ser Ile Thr Ser Val Ser Ile Leu Asp Cys Trp	
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aca atc gtg tgg tcc atc atc ttt aca tgg atg ttc cta ggc aca aaa	683
Thr Ile Val Trp Ser Ile Ile Phe Thr Trp Met Phe Leu Gly Thr Lys	
115 120 125 130	
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Tyr Ser Val Tyr Gln Phe Val Gly Ala Ala Ile Cys Val Gly Gly Leu	
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Leu Leu Val Leu Leu Ser Asp Ser Gly Val Thr Ala Ala Gly Ser Asn	
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Pro Leu Leu Gly Asp Phe Leu Val Ile Thr Gly Ser Ile Leu Phe Thr	
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Leu Ser Thr Val Gly Gln Glu Tyr Cys Val Lys Arg Lys Asp Arg Ile	
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Thr Gly Leu Leu Ala Ala Tyr Val Val Tyr Ala Leu Ser Ser Phe Leu	
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Phe Cys Thr Leu Thr Pro Phe Leu Leu Lys Met Ser Gly Ala Ala Phe	
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 310 315 320  
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 aaaacataca agttttttaat ttttactaa gcaagaaaat atg gtg cag cct cat gtc 358  
 Met Val Gln Pro His Val  
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 atc tta aca aca ttt cca gca caa ggc cat att aat cca gca ctt caa 406  
 Ile Leu Thr Thr Phe Pro Ala Gln Gly His Ile Asn Pro Ala Leu Gln  
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Phe	Asp	His	Ser	Lys	Asp	Pro	Val	Phe	Tyr	Met	Ser	Gln	Leu	Arg	Lys	
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Cys	Gly	Ser	Glu	Thr	Val	Lys	Lys	Ile	Ile	Leu	Thr	Cys	Ser	Glu	Asn	
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Gly	Gln	Pro	Ile	Thr	Cys	Leu	Leu	Tyr	Ser	Ile	Phe	Leu	Pro	Trp	Ala	
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Ala	Glu	Val	Ala	Arg	Glu	Val	His	Ile	Pro	Ser	Ala	Leu	Leu	Trp	Ser	
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Gln	Pro	Ala	Thr	Ile	Leu	Asp	Ile	Tyr	Tyr	Phe	Asn	Phe	His	Gly	Tyr	
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Leu	Pro	Gly	Leu	Pro	Leu	Leu	Glu	Thr	Arg	Asp	Leu	Pro	Ser	Phe	Leu	
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Leu	Pro	Tyr	Gly	Ala	Lys	Gly	Ser	Leu	Arg	Val	Ala	Leu	Pro	Pro	Phe	
		185					190					195				
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Lys	Glu	Leu	Ile	Asp	Thr	Leu	Asp	Ala	Glu	Thr	Thr	Pro	Lys	Ile	Leu	
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Val	Asn	Thr	Phe	Asp	Glu	Leu	Glu	Pro	Glu	Ala	Leu	Asn	Ala	Ile	Glu	
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Gly	Tyr	Lys	Phe	Tyr	Gly	Ile	Gly	Pro	Leu	Ile	Pro	Ser	Ala	Phe	Leu	
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Trp Val Ile Lys Glu Asn Glu Lys Gly Lys Glu Glu Glu Asn Lys Lys	
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Cys Ser Gln Leu Glu Val Leu Lys His Pro Ser Leu Gly Cys Phe Val	
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395 400 405	
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Asp Gly Val Val Glu Ser Glu Glu Ile Lys Arg Cys Ile Glu Leu Val	
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Met Asp Gly Gly Glu Lys Gly Glu Glu Leu Arg Lys Asn Ala Lys Lys	
425 430 435	

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 115 120 125  
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Pro	Ser	Glu	Thr	Ser	Tyr	Gly	Gly	Asp	Leu	Phe	Glu	Lys	Ser	Glu	Glu
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Glu	Ile	Gly	Lys	Gly	Leu	Leu	Ala	Cys	Gly	Arg	Pro	Phe	Leu	Trp	Met
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Ile	Arg	Glu	Gln	Lys	Asn	Asp	Asp	Gly	Glu	Glu	Glu	Glu	Glu	Glu	Leu
305				310					315						320
Ser	Cys	Ile	Gly	Glu	Leu	Lys	Lys	Met	Gly	Lys	Ile	Val	Ser	Trp	Cys
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Ser	Gln	Leu	Glu	Val	Leu	Ala	His	Pro	Ala	Leu	Gly	Cys	Phe	Val	Thr
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His	Cys	Gly	Trp	Asn	Ser	Ala	Val	Glu	Ser	Leu	Ser	Cys	Gly	Val	Pro
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		370				375					380				
Ile	Glu	Asp	Ala	Trp	Gly	Thr	Gly	Val	Arg	Val	Arg	Met	Asn	Glu	Gly
385				390					395					400	
Gly	Gly	Val	Asp	Gly	Ser	Glu	Ile	Glu	Arg	Cys	Val	Glu	Met	Val	Met
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Asp Gly Gly Glu Lys Ser Lys Leu Val Arg Glu Asn Ala Ile Lys Trp  
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 Lys Thr Leu Ala Arg Glu Ala Met Gly Glu Asp Gly Ser Ser Leu Lys  
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                   35                                  40                                  45  
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                   50                                  55                                  60  
 Phe Ser Asp Gly Tyr Asp Asp Gly Leu Lys Pro Gly Gly Asp Gly Lys  
       65                                  70                                  75                                  80  
 Arg Tyr Met Ser Glu Met Lys Ala Arg Gly Ser Glu Ala Leu Arg Asn  
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 Leu Leu Leu Asn Asn Asp Asp Val Thr Phe Val Val Tyr Ser His Leu  
                   100                                  105                                  110  
 Phe Ala Trp Ala Ala Glu Val Ala Arg Leu Ser His Val Pro Thr Ala  
                   115                                  120                                  125  
 Leu Leu Trp Val Glu Pro Ala Thr Val Leu Cys Ile Tyr His Phe Tyr  
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 Phe Asn Gly Tyr Ala Asp Glu Ile Asp Ala Gly Ser Asn Glu Ile Gln  
       145                                  150                                  155                                  160  
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 Leu Pro Ala Thr Pro Glu Arg Phe Arg Leu Met Met Lys Glu Lys Leu  
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Glu Thr Leu Asp Gly Glu Glu Lys Ala Lys Val Leu Val Asn Thr Phe  
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 Pro Ser Glu Thr Ser Tyr Gly Gly Asp Leu Phe Glu Lys Ser Glu Glu  
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 Ser Glu Met Lys Ser Arg Gly Ile Lys Ala Leu Ser Asp Thr Leu Ala  
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 Tyr Phe Tyr Phe Asn Gly Tyr Ser Asp Glu Ile Asp Ala Gly Ser Asp  
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 Lys Glu Lys Leu Glu Thr Leu Glu Gly Glu Glu Lys Pro Lys Val Leu  
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 Val Asn Ser Phe Asp Ala Leu Glu Pro Asp Ala Leu Lys Ala Ile Asp  
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 Lys Tyr Glu Met Ile Ala Ile Gly Pro Leu Ile Pro Ser Ala Phe Leu  
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 Asp Gly Lys Asp Pro Ser Asp Arg Ser Phe Gly Gly Asp Leu Phe Glu  
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 Lys Gly Ser Asn Asp Asp Asp Cys Leu Glu Trp Leu Ser Thr Asn Pro  
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Arg Ser Ser Val Val Tyr Val Ser Phe Gly Ser Phe Val Asn Thr Thr  
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 Lys Ser Gln Met Glu Glu Ile Ala Arg Gly Leu Leu Asp Cys Gly Arg  
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 Met Asp Gly Gly Glu Lys Ser Arg Lys Leu Arg Glu Ser Ala Gly Lys  
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Phe	Arg	Thr	Leu	Met	Lys	Glu	Lys	Phe	Asp	Thr	Leu	Asp	Lys	Glu	Pro			
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 Cys Ser Gln Leu Asp Val Leu Thr His Lys Ser Val Gly Cys Phe Val  
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 Asn Lys Ala Phe Glu Leu Thr Ser Ile Thr Ser Val Ser Ile Leu Asp  
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 Trp Ser Thr Gly Leu Leu Ala Ala Tyr Val Val Tyr Ala Leu Ser Ser  
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Phe Asn Phe His Gly Tyr Glu Lys Ala Met Ala Asn Glu Ser Asn Asp  
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Asp Leu Pro Ser Phe Leu Leu Pro Tyr Gly Ala Lys Gly Ser Leu Arg  
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Val Ala Leu Pro Pro Phe Lys Glu Leu Ile Asp Thr Leu Asp Ala Glu  
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 Arg Lys Asn Ala Lys Lys Trp Lys Glu Leu Ala Arg Glu Ala Val Lys  
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Sequence

Sequence ID No.: 1  
Sequence length: 1507  
Sequence type: Nucleic acid  
Number of strands: Double-strand  
Topology: Straight chain  
Source:

Biological name: Perilla (Perilla frutescens)

Tissue type: Leaf

Direct source:

Library name: cDNA library

Clone name: p3R4

Sequence:

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1 5 10																
CCT	GCG	CAA	GGC	CAC	ATA	AAT	CCC	GCC	CTC	CAA	TTC	GCC	AAG	AGA	CTC	97
Pro	Ala	Gln	Gly	His	Ile	Asn	Pro	Ala	Leu	Gln	Phe	Ala	Lys	Arg	Leu	
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CTA	AAA	GCC	GGC	ACT	GAC	GTC	ACA	TTT	TTC	ACG	AGC	GTT	TAT	GCA	TGG	145
Leu	Lys	Ala	Gly	Thr	Asp	Val	Thr	Phe	Phe	Thr	Ser	Val	Tyr	Ala	Trp	
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CGC	CGC	ATG	GCC	AAC	ACA	GCC	TCC	GCC	GCT	GCC	GGA	AAC	CCA	CCG	GGC	193
Arg	Arg	Met	Ala	Asn	Thr	Ala	Ser	Ala	Ala	Ala	Gly	Asn	Pro	Pro	Gly	
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CTC	GAC	TTC	GTG	GCG	TTC	TCC	GAC	GGC	TAC	GAC	GAC	GGG	CTG	AAG	CCC	241
Leu	Asp	Phe	Val	Ala	Phe	Ser	Asp	Gly	Tyr	Asp	Asp	Gly	Leu	Lys	Pro	
60 65 70 75																
TGC	GGC	GAC	GGG	AAG	CGC	TAC	ATG	TCC	GAG	ATG	AAA	GCC	CGC	GGC	TCC	289
Cys	Gly	Asp	Gly	Lys	Arg	Tyr	Met	Ser	Glu	Met	Lys	Ala	Arg	Gly	Ser	
80 85 90																
GAG	GCC	TTA	AGA	AAC	CTC	CTT	CTC	AAC	AAC	CAC	GAC	GTC	ACG	TTC	GTC	337
Glu	Ala	Leu	Arg	Asn	Leu	Leu	Leu	Asn	Asn	His	Asp	Val	Thr	Phe	Val	
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Val Tyr Ser His Leu Phe Ala Trp Ala Ala Glu Val Ala Arg Glu Ser	
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Gln Val Pro Ser Ala Leu Leu Trp Val Glu Pro Ala Thr Val Leu Cys	
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Ser Asp Glu Ile Gln Leu Pro Arg Leu Pro Pro Leu Glu Gln Arg Ser	
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CTT CCG ACC TTT CTG CTG CCG GAG ACA CCG GAG AGA TTC CGG TTG ATG	577
Leu Pro Thr Phe Leu Leu Pro Glu Thr Pro Glu Arg Phe Arg Leu Met	
175 180 185	
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190 195 200	
TTG GTG AAC ACG TTT GAT GCG TTG GAG CCC GAT GCA CTC ACG GCT ATT	673
Leu Val Asn Thr Phe Asp Ala Leu Glu Pro Asp Ala Leu Thr Ala Ile	
205 210 215	
GAT AGG TAT GAG TTG ATC GGG ATC GGG CCG TTG ATT CCC TCC GCC TTC	721
Asp Arg Tyr Glu Leu Ile Gly Ile Gly Pro Leu Ile Pro Ser Ala Phe	
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TTG GAC GGC GGA GAT CCC TCC GAA ACG TCT TAC GGC GGC GAT CTT TTC	769
Leu Asp Gly Gly Asp Pro Ser Glu Thr Ser Tyr Gly Gly Asp Leu Phe	
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GAA AAA TCG GAG GAG AAT AAC TGC GTG GAG TGG TTG GAC ACG AAG CCG	817
Glu Lys Ser Glu Glu Asn Asn Cys Val Glu Trp Leu Asp Thr Lys Pro	
255 260 265	
AAA TCT TCG GTG GTG TAT GTG TCG TTT GGG AGC GTT TTG AGG TTT CCA	865
Lys Ser Ser Val Val Tyr Val Ser Phe Gly Ser Val Leu Arg Phe Pro	
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Lys Ala Gln Met Glu Glu Ile Gly Lys Gly Leu Leu Ala Cys Gly Arg	
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ATA GTT TCG TGG TGC TCG CAG TTG GAG GTT CTG GCG CAC CCT GCG TTG      1057
Ile Val Ser Trp Cys Ser Gln Leu Glu Val Leu Ala His Pro Ala Leu
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Gly Cys Phe Val Thr His Cys Gly Trp Asn Ser Ala Val Glu Ser Leu
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AGT TGC GGG GTT CCG GTG GTG GCG GTG CCG CAG TGG TTT GAT CAG ACG      1153
Ser Cys Gly Val Pro Val Val Ala Val Pro Gln Trp Phe Asp Gln Thr
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ACG AAT GCG AAG CTG ATT GAG GAT GCG TGG GGG ACA GGG GTG AGA GTG      1201
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AGA ATG AAT GAA GGG GGT GGG GTT GAT GGA TCT GAG ATA GAG AGG TGT      1249
Arg Met Asn Glu Gly Gly Gly Val Asp Gly Ser Glu Ile Glu Arg Cys
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GTG GAG ATG GTG ATG GAT GGG GGT GAG AAG AGC AAA CTA GTG AGA GAA      1297
Val Glu Met Val Met Asp Gly Gly Glu Lys Ser Lys Leu Val Arg Glu
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AAT GCC ATA AAA TGG AAG ACT TTG GCC AGA GAA GCC ATG GGA GAG GAT      1345
Asn Ala Ile Lys Trp Lys Thr Leu Ala Arg Glu Ala Met Gly Glu Asp
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GGA TCT TCA CTC AAG AAT CTC AAC GCC TTT CTT CAT CAA GTT GCA CGT      1393
Gly Ser Ser Leu Lys Asn Leu Asn Ala Phe Leu His Gln Val Ala Arg
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GCT TAATACACAA AATGGCTTTC CACTTTTAAT CTACTCAAAC ACCGGTTCAA      1446
Ala
460                                                                1507
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Sequence ID No.: 2

Sequence length: 1470

Sequence type: Nucleic acid

Number of strands: Double-strand

Topology: Straight chain

Source:

Biological name: Perilla (Perilla frutescens)

Tissue type: Leaf

Direct source:

Library name: cDNA library

Clone name: p3R6

Sequence:

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Ala Thr Phe Pro Ala Gln Gly His Ile Asn Pro Ala Leu Gln Phe Ala	
10 15 20	
AAG AGA CTC CTA AAA GCC GGC ACT GAC GTC ACG TTT TTC ACG AGC GTT	144
Lys Arg Leu Leu Lys Ala Gly Thr Asp Val Thr Phe Phe Thr Ser Val	
25 30 35 40	
TAT GCA TGG CGC CGC ATG GCC AAC ACA GCC TCC GCC GCT GCC GGA AAC	192
Tyr Ala Trp Arg Arg Met Ala Asn Thr Ala Ser Ala Ala Ala Gly Asn	
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Pro Pro Gly Leu Asp Phe Val Ala Phe Ser Asp Gly Tyr Asp Asp Gly	
60 65 70	
CTG AAG CCC GGC GGC GAC GGG AAG CGC TAC ATG TCC GAG ATG AAA GCC	288
Leu Lys Pro Gly Gly Asp Gly Lys Arg Tyr Met Ser Glu Met Lys Ala	
75 80 85	
CGC GGC TCC GAG GCC TTA AGA AAC CTC CTT CTC AAC AAC GAC GAC GTC	336
Arg Gly Ser Glu Ala Leu Arg Asn Leu Leu Leu Asn Asn Asp Asp Val	
90 95 100	
ACT TTC GTC GTC TAC TCC CAC CTC TTT GCA TGG GCG GCG GAG GTG GCG	384
Thr Phe Val Val Tyr Ser His Leu Phe Ala Trp Ala Ala Glu Val Ala	
105 110 115 120	

CGT TTG TCC CAC GTC CCG ACC GCC CTT CTC TGG GTC GAG CCC GCC ACC	432
Arg Leu Ser His Val Pro Thr Ala Leu Leu Trp Val Glu Pro Ala Thr	
125 130 135	
GTG CTG TGC ATA TAC CAC TTC TAC TTC AAC GGC TAC GCA GAC GAG ATC	480
Val Leu Cys Ile Tyr His Phe Tyr Phe Asn Gly Tyr Ala Asp Glu Ile	
140 145 150	
GAC GCC GGT TCC AAT GAA ATT CAG CTC CCT CGG CTT CCA TCC CTG GAG	528
Asp Ala Gly Ser Asn Glu Ile Gln Leu Pro Arg Leu Pro Ser Leu Glu	
155 160 165	
CAG CGC AGT CTT CCG ACG TTT CTG CTG CCT GCG ACG CCG GAG AGA TTC	576
Gln Arg Ser Leu Pro Thr Phe Leu Leu Pro Ala Thr Pro Glu Arg Phe	
170 175 180	
CGG TTG ATG ATG AAG GAG AAG CTG GAA ACT TTA GAC GGT GAA GAG AAG	624
Arg Leu Met Met Lys Glu Lys Leu Glu Thr Leu Asp Gly Glu Glu Lys	
185 190 195 200	
GCG AAA GTA TTG GTG AAC ACG TTT GAT GCG TTG GAG CCC GAT GCA CTC	672
Ala Lys Val Leu Val Asn Thr Phe Asp Ala Leu Glu Pro Asp Ala Leu	
205 210 215	
ACG GCT ATT GAT AGG TAT GAG TTG ATC GGG ATC GGG CCG TTG ATT CCC	720
Thr Ala Ile Asp Arg Tyr Glu Leu Ile Gly Ile Gly Pro Leu Ile Pro	
220 225 230	
TCC GCC TTC TTG GAC GGC GAA GAT CCC TCC GAA ACG TCT TAC GGC GGC	768
Ser Ala Phe Leu Asp Gly Glu Asp Pro Ser Glu Thr Ser Tyr Gly Gly	
235 240 245	
GAT CTT TTC GAA AAA TCG GAG GAG AAT AAC TGC GTG GAG TGG TTG AAC	816
Asp Leu Phe Glu Lys Ser Glu Glu Asn Asn Cys Val Glu Trp Leu Asn	
250 255 260	
TCG AAG CCG AAA TCT TCG GTG GTG TAT GTG TCG TTT GGG AGC GTT TTG	864
Ser Lys Pro Lys Ser Ser Val Val Tyr Val Ser Phe Gly Ser Val Leu	
265 270 275 280	
AGG TTT CCA AAG GCA CAA ATG GAA GAG ATT GGG AAA GGG CTA TTA GCC	912
Arg Phe Pro Lys Ala Gln Met Glu Glu Ile Gly Lys Gly Leu Leu Ala	
285 290 295	
TGC GGA AGG CCC TTT TTA TGG ATG ATA CGA GAA CAG AAG AAT GAC GAC	960
Cys Gly Arg Pro Phe Leu Trp Met Ile Arg Glu Gln Lys Asn Asp Asp	
300 305 310	

CGT TTG TCC CAC GTC CCG ACC GCC CTT CTC TGG GTC GAG CCC GCC ACC

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GGC GAA GAA GAA GAA GAA GAA GAA GAG TTG AGT TGC ATT GGG GAA TTG 1008
Gly Glu Glu Glu Glu Glu Glu Glu Glu Leu Ser Cys Ile Gly Glu Leu
      315              320              325
AAA AAA ATG GGG AAA ATA GTG TCG TGG TGC TCG CAG TTG GAG GTT CTG 1056
Lys Lys Met Gly Lys Ile Val Ser Trp Cys Ser Gln Leu Glu Val Leu
      330              335              340
GCG CAC CCT GCG TTG GGA TGT TTC GTG ACG CAT TGT GGG TGG AAC TCG 1104
Ala His Pro Ala Leu Gly Cys Phe Val Thr His Cys Gly Trp Asn Ser
345              350              355              360
GCT GTG GAG AGC TTG AGT TGC GGG ATT CCG GTG GTG GCG GTG CCG CAG 1152
Ala Val Glu Ser Leu Ser Cys Gly Ile Pro Val Val Ala Val Pro Gln
      365              370              375
TGG TTT GAT CAG ACG ACG AAT GCG AAG CTG ATT GAG GAT GCG TGG GGG 1200
Trp Phe Asp Gln Thr Thr Asn Ala Lys Leu Ile Glu Asp Ala Trp Gly
      380              385              390
ACA GGG GTG AGA GTG AGA ATG AAT GAA GGG GGT GGG GTT GAT GGA TGT 1248
Thr Gly Val Arg Val Arg Met Asn Glu Gly Gly Gly Val Asp Gly Cys
      395              400              405
GAG ATA GAA AGG TGT GTG GAG ATG GTG ATG GAT GGG GGT GAC AAG ACC 1296
Glu Ile Glu Arg Cys Val Glu Met Val Met Asp Gly Gly Asp Lys Thr
      410              415              420
AAA CTA GTG AGA GAA AAT GCC ATC AAA TGG AAG ACT TTG GCC AGA CAA 1344
Lys Leu Val Arg Glu Asn Ala Ile Lys Trp Lys Thr Leu Ala Arg Gln
425              430              435              440
GCC ATG GGA TAGGATGGAT CTTCACTCAA CAATCTCAAC GCCTTTCTTC 1393
Ala Met Gly
      443
GTCAAGTTGC ACACTTTTAA TCTGCTCAAA CAGCGGTTCA AATAAATATC CCCTTCCACT 1453
TAAAAAAAAA AAAAAAA 1470

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Sequence ID No.: 3

Sequence length: 2062

Sequence type: Nucleic acid

Number of strands: Double-strand

Topology: Straight chain

Source:

Biological name: Verbena (Verbena hybrida)

Tissue type: Petal

Clone name: pSHGT8

ATTTTACCAA AAAAATAAAA AAAAA ATG AGC AGA GCT CAC GTC CTC TTG GCC 52

1 5

Thr Phe Pro Ala Gln Gly His Ile Asn Pro Ala Leu Gln Phe Ala Lys

10                      15                      20                      25

Arg Leu Ala Asn Ala Asp Ile Gln Val Thr Phe Phe Thr Ser Val Tyr

30                      35                      40

Ala Trp Arg Arg Met Ser Arg Thr Ala Ala Gly Ser Asn Gly Leu Ile

45                      50                      55

Asn Phe Val Ser Phe Ser Asp Gly Tyr Asp Asp Gly Leu Gln Pro Gly

60                      65                      70

Asp Asp Gly Lys Asn Tyr Met Ser Glu Met Lys Ser Arg Gly Ile Lys

75                      80                      85

Ala Leu Ser Asp Thr Leu Ala Ala Asn Asn Val Asp Gln Lys Ser Ser

90                      95                      100                      105

Lys Ile Thr Phe Val Val Tyr Ser His Leu Phe Ala Trp Ala Ala Lys

110                      115                      120

Val Ala Arg Glu Phe His Leu Arg Ser Ala Leu Leu Trp Ile Glu Pro

125                      130                      135

Ala Thr Val Leu Asp Ile Phe Tyr Phe Tyr Phe Asn Gly Tyr Ser Asp

140                      145                      150



GAA ATC GAT GCG GGT TCG GAT GCT ATT CAC TTG CCC GGA GGA CTC CCA	532
Glu Ile Asp Ala Gly Ser Asp Ala Ile His Leu Pro Gly Gly Leu Pro	
155 160 165	
GTG CTG GCC CAG CGT GAT TTA CCG TCT TTC CTT CTT CCT TCC ACG CAT	580
Val Leu Ala Gln Arg Asp Leu Pro Ser Phe Leu Leu Pro Ser Thr His	
170 175 180 185	
GAG AGA TTC CGT TCA CTG ATG AAG GAG AAA TTG GAA ACT TTA GAA GGT	628
Glu Arg Phe Arg Ser Leu Met Lys Glu Lys Leu Glu Thr Leu Glu Gly	
190 195 200	
GAA GAA AAA CCT AAG GTC TTG GTG AAC AGC TTT GAT GCG TTG GAG CCT	676
Glu Glu Lys Pro Lys Val Leu Val Asn Ser Phe Asp Ala Leu Glu Pro	
205 210 215	
GAT GCG CTC AAG GCC ATT GAT AAG TAC GAG ATG ATT GCA ATC GGG CCG	724
Asp Ala Leu Lys Ala Ile Asp Lys Tyr Glu Met Ile Ala Ile Gly Pro	
220 225 230	
TTG ATT CCT TCC GCA TTC TTG GAC GGT AAA GAT CCT TCG GAC AGG TCT	772
Leu Ile Pro Ser Ala Phe Leu Asp Gly Lys Asp Pro Ser Asp Arg Ser	
235 240 245	
TTC GGC GGA GAT TTG TTC GAG AAA GGG TCG AAT GAC GAC GAT TGC CTC	820
Phe Gly Gly Asp Leu Phe Glu Lys Gly Ser Asn Asp Asp Asp Cys Leu	
250 255 260 265	
GAA TGG TTG AGC ACG AAT CCT CGA TCT TCG GTG GTT TAC GTT TCG TTC	868
Glu Trp Leu Ser Thr Asn Pro Arg Ser Ser Val Val Tyr Val Ser Phe	
270 275 280	
GGA AGC TTC GTT AAT ACG ACG AAG TCG CAA ATG GAA GAG ATA GCA AGA	916
Gly Ser Phe Val Asn Thr Thr Lys Ser Gln Met Glu Glu Ile Ala Arg	
285 290 295	
GGG CTG TTA GAT TGT GGG AGG CCG TTT TTG TGG GTG GTA AGA GTA AAC	964
Gly Leu Leu Asp Cys Gly Arg Pro Phe Leu Trp Val Val Arg Val Asn	
300 305 310	
GAA GGA GAA GAG GTA TTG ATA AGT TGC ATG GAG GAG TTG AAA CGA GTG	1012
Glu Gly Glu Glu Val Leu Ile Ser Cys Met Glu Glu Leu Lys Arg Val	
315 320 325	
GGG AAA ATT GTA TCT TGG TGT TCT CAA TTG GAA GTC CTG ACG CAT CCC	1060
Gly Lys Ile Val Ser Trp Cys Ser Gln Leu Glu Val Leu Thr His Pro	
330 335 340 345	

TCG TTG GGA TGT TTC GTG ACA CAC TGC GGG TGG AAT TCG ACT CTA GAG	1108
Ser Leu Gly Cys Phe Val Thr His Cys Gly Trp Asn Ser Thr Leu Glu	
350 355 360	
AGT ATA TCT TTC GGG GTT CCG ATG GTG GCT TTT CCG CAG TGG TTC GAT	1156
Ser Ile Ser Phe Gly Val Pro Met Val Ala Phe Pro Gln Trp Phe Asp	
365 370 375	
CAA GGG ACG AAT GCG AAG CTG ATG GAG GAT GTG TGG AGG ACG GGT GTG	1204
Gln Gly Thr Asn Ala Lys Leu Met Glu Asp Val Trp Arg Thr Gly Val	
380 385 390	
AGA GTG AGA GCT AAT GAG GAG GGT AGC GTC GTT GAT GGT GAT GAA ATT	1252
Arg Val Arg Ala Asn Glu Glu Gly Ser Val Val Asp Gly Asp Glu Ile	
395 400 405	
AGG AGA TGT ATT GAG GAG GTT ATG GAT GGG GGA GAA AAG AGT AGG AAA	1300
Arg Arg Cys Ile Glu Glu Val Met Asp Gly Gly Glu Lys Ser Arg Lys	
410 415 420 425	
CTT AGA GAG AGT GCT GGC AAG TGG AAG GAT TTG GCA AGA AAA GCT ATG	1348
Leu Arg Glu Ser Ala Gly Lys Trp Lys Asp Leu Ala Arg Lys Ala Met	
430 435 440	
GAG GAA GAT GGA TCT TCA GTT AAC AAC CTC AAG GTC TTT CTT GAT GAG	1396
Glu Glu Asp Gly Ser Ser Val Asn Asn Leu Lys Val Phe Leu Asp Glu	
445 450 455	
GTT GTA GGT ATC TAAAGACGTA AATGAGGTCC CCATAGGCAA AATTGCAAAT	1448
Val Val Gly Ile	
460 461	
TTCATCTCGT AAGTTGAATA CTTTTTGGCT TTAATTTTGT TCGAGTTTGT TTTTCAAAT	1508
TTATCTTGTA ATTTTACATT GAGTGTAAT TTAGTCTGAT TTAACTGGA AAAATATAAA	1568
ATTCATTGTT GAGACTCTTC ATCAAAATCA TCTGATTTCC TTTATTGTCT TGGTCAAAT	1628
TCTCATATCA ATTGGAAAAA ATAAATTTCA AAATCGTCCA ATTTTGAACC AAGAAAGAAG	1688
TATAATTTGA CCAAAATAAT AAAAGGATTC AAGTGATCTT GATGAAGTGT CTGAGCGACG	1748
AGTTCTATAT TTTTCCACCG AATTTCTAAC GAGTTTTTGA ATTTTTTTTA GCCAAAATCG	1808
GACTAACTTT GTACAAAATG AAAAGTTATA TGATGAAATT TTAAAAACA AACTCAGACA	1868
ATAATAAAGC CCGAAAGTAG TAAAATTACC TGACGAAATT TGCAATTTTCG CCTCCTATTT	1928
TAATTTTTTTT GGTGTGTTTA ATAAATCGGT TATTTTACTT TTAATTAAAA TAAAAGTGAG	1988
ATGCATGATA GCTTGGTGAG TATATATGAG TTGATGGTAA TGTACGATAT TTTCTAAAAA	2048
AAAAAAAAAA AAAA	2062

Sequence ID No.: 4

Sequence length: 1671

Sequence type: Nucleic acid  
Number of strands: Double-strand  
Topology: Straight chain  
Source:

Biological name: Trenia

Tissue type: Petal

Direct source:

Library name: cDNA library

Clone name: pSTGT5

Sequence:

```
AACACATAAA AAAAAAATAA AAGAAGAAAT AATTAAAAAA AAAA ATG GTT AAC      53
                                     Met Val Asn
                                     1

AAA CGC CAT ATT CTA CTA GCA ACA TTC CCA GCA CAA GGC CAC ATA AAC      101
Lys Arg His Ile Leu Leu Ala Thr Phe Pro Ala Gln Gly His Ile Asn
      5              10              15

CCT TCT CTC GAG TTC GCC AAA AGG CTC CTC AAC ACC GGA TAC GTC GAC      149
Pro Ser Leu Glu Phe Ala Lys Arg Leu Leu Asn Thr Gly Tyr Val Asp
      20              25              30              35

CAA GTC ACA TTC TTC ACG AGT GTA TAC GCA TTG AGA CGC ATG CGC TTC      197
Gln Val Thr Phe Phe Thr Ser Val Tyr Ala Leu Arg Arg Met Arg Phe
              40              45              50

GAA ACC GAT CCG AGC AGC AGA ATC GAT TTC GTG GCA TKT YCA GAT TCT      245
Glu Thr Asp Pro Ser Ser Arg Ile Asp Phe Val Ala Xaa Xaa Asp Ser
              55              60              65

TAC GAT GAT GGC TTA AAG AAA GGC GAC GAT GGC AAA AAC TAC ATG TCG      293
Tyr Asp Asp Gly Leu Lys Lys Gly Asp Asp Gly Lys Asn Tyr Met Ser
              70              75              80

GAG ATG AGA AAG CGC GGA ACG AAG GCC TTA AAG GAC ACT CTT ATT AAG      341
Glu Met Arg Lys Arg Gly Thr Lys Ala Leu Lys Asp Thr Leu Ile Lys
              85              90              95

CTC AAC GAT GCT GCG ATG GGA AGT GAA TGT TAC AAT CGC GTG AGC TTT      389
Leu Asn Asp Ala Ala Met Gly Ser Glu Cys Tyr Asn Arg Val Ser Phe
100              105              110              115
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GTG	GTG	TAC	TCT	CAT	CTA	TTT	TCG	TGG	GCA	GCT	GAA	GTG	GCG	CGT	GAA	437
Val	Val	Tyr	Ser	His	Leu	Phe	Ser	Trp	Ala	Ala	Glu	Val	Ala	Arg	Glu	
				120					125					130		
GTC	GAC	GTG	CCG	AGT	GCC	CTT	CTT	TGG	ATT	GAA	CCG	GCT	ACG	GTT	TTC	485
Val	Asp	Val	Pro	Ser	Ala	Leu	Leu	Trp	Ile	Glu	Pro	Ala	Thr	Val	Phe	
			135					140					145			
GAT	GTG	TAC	TAT	TTT	TAC	TTC	AAT	GGG	TAT	GCC	GAT	GAT	ATC	GAT	GCG	533
Asp	Val	Tyr	Tyr	Phe	Tyr	Phe	Asn	Gly	Tyr	Ala	Asp	Asp	Ile	Asp	Ala	
		150					155					160				
GGC	TCA	GAT	CAA	ATC	CAA	CTG	CCC	AAT	CTT	CCG	CAG	CTC	TCC	AAG	CAA	581
Gly	Ser	Asp	Gln	Ile	Gln	Leu	Pro	Asn	Leu	Pro	Gln	Leu	Ser	Lys	Gln	
	165					170					175					
GAT	CTC	CCC	TCT	TTC	CTA	CTC	CCT	TCG	AGC	CCC	GCG	AGA	TTC	CGA	ACC	629
Asp	Leu	Pro	Ser	Phe	Leu	Leu	Pro	Ser	Ser	Pro	Ala	Arg	Phe	Arg	Thr	
180					185				190					195		
CTA	ATG	AAA	GAA	AAG	TTC	GAC	ACG	CTC	GAC	AAA	GAA	CCG	AAA	GCG	AAG	677
Leu	Met	Lys	Glu	Lys	Phe	Asp	Thr	Leu	Asp	Lys	Glu	Pro	Lys	Ala	Lys	
			200					205					210			
GTC	TTG	ATA	AAC	ACG	TTC	GAC	GCA	TTA	GAA	ACC	GAA	CAA	CTC	AAA	GCC	725
Val	Leu	Ile	Asn	Thr	Phe	Asp	Ala	Leu	Glu	Thr	Glu	Gln	Leu	Lys	Ala	
			215					220					225			
ATC	GAC	AGG	TAT	GAA	CTA	ATA	TCC	ATC	GGC	CCA	TTA	ATC	CCA	TCA	TCG	773
Ile	Asp	Arg	Tyr	Glu	Leu	Ile	Ser	Ile	Gly	Pro	Leu	Ile	Pro	Ser	Ser	
		230					235					240				
ATA	TTC	TCA	GAT	GGC	AAC	GAC	CCC	TCA	TCA	AGC	AAC	AAA	TCC	TAC	GGT	821
Ile	Phe	Ser	Asp	Gly	Asn	Asp	Pro	Ser	Ser	Ser	Asn	Lys	Ser	Tyr	Gly	
	245					250					255					
GGA	GAC	CTC	TTC	AGA	AAA	GCC	GAT	GAA	ACT	TAC	ATG	GAC	TGG	CTA	AAC	869
Gly	Asp	Leu	Phe	Arg	Lys	Ala	Asp	Glu	Thr	Tyr	Met	Asp	Trp	Leu	Asn	
260					265					270				275		
TCA	AAA	CCC	GAA	TCA	TCG	GTC	GTT	TAC	GTT	TCG	TTC	GGG	AGC	CTC	CTG	917
Ser	Lys	Pro	Glu	Ser	Ser	Val	Val	Tyr	Val	Ser	Phe	Gly	Ser	Leu	Leu	
			280					285					290			
AGG	CTC	CCG	AAA	CCC	CAA	ATG	GAA	GAA	ATA	GCA	ATA	GGG	CTT	TCA	GAC	965
Arg	Leu	Pro	Lys	Pro	Gln	Met	Glu	Glu	Ile	Ala	Ile	Gly	Leu	Ser	Asp	
			295					300					305			

ACC AAA TCG CCA GTT CTC TGG GTG ATA AGA AGA AAC GAA GAG GGC GAC 1013  
Thr Lys Ser Pro Val Leu Trp Val Ile Arg Arg Asn Glu Glu Gly Asp  
310 315 320  
GAA CAA GAG CAA GCA GAA GAA GAA GAG AAG CTG CTG AGC TTC TTT GAT 1061  
Glu Gln Glu Gln Ala Glu Glu Glu Glu Lys Leu Leu Ser Phe Phe Asp  
325 330 335  
CGT CAC GGA ACT GAA CGA CTC GGG AAA ATC GTG ACA TGG TGC TCA CAA 1109  
Arg His Gly Thr Glu Arg Leu Gly Lys Ile Val Thr Trp Cys Ser Gln  
340 345 350 355  
TTG GAT GTT CTG ACG CAT AAG TCG GTG GGA TGC TTC GTG ACG CAT TGC 1157  
Leu Asp Val Leu Thr His Lys Ser Val Gly Cys Phe Val Thr His Cys  
360 365 370  
GGT TGG AAT TCT GCT ATC GAG AGC CTG GCT TGT GGT GTG CCC GTG GTG 1205  
Gly Trp Asn Ser Ala Ile Glu Ser Leu Ala Cys Gly Val Pro Val Val  
375 380 385  
TGC TTT CCT CAA TGG TTC GAT CAA GGG ACT AAT GCG AAG ATG ATC GAA 1253  
Cys Phe Pro Gln Trp Phe Asp Gln Gly Thr Asn Ala Lys Met Ile Glu  
390 395 400  
GAT GTG TGG AGG AGT GGT GTG AGA GTC AGA GTG AAT GAG GAA GGC GGC 1301  
Asp Val Trp Arg Ser Gly Val Arg Val Arg Val Asn Glu Glu Gly Gly  
405 410 415  
GTT GTT GAT AGG CGT GAG ATT AAG AGG TGC GTC TCG GAG GTT ATA AAG 1349  
Val Val Asp Arg Arg Glu Ile Lys Arg Cys Val Ser Glu Val Ile Lys  
420 425 430 435  
AGT CGA GAG TTG AGA GAA AGC GCA ATG ATG TGG AAG GGT TTG GCT AAA 1397  
Ser Arg Glu Leu Arg Glu Ser Ala Met Met Trp Lys Gly Leu Ala Lys  
440 445 450  
GAA GCT ATG GAT GAA GAA CGT GGA TCA TCA ATG AAC AAT CTG AAG AAT 1445  
Glu Ala Met Asp Glu Glu Arg Gly Ser Ser Met Asn Asn Leu Lys Asn  
455 460 465  
TTT ATT ACT AGG ATT ATT AAT GAA AAT GCC TCA TAAGTTGTAC 1488  
Phe Ile Thr Arg Ile Ile Asn Glu Asn Ala Ser  
470 475 478  
TATATATGTT ATTATTGTTG TTATGGACGT CGAATTAAGT ATTAGTTAAA TGATATGTAT 1548  
TTAGAGGAAG GCCAAAACGG GCTACACCCG GCAGGCCACG GGTTGGAAAA GCCCGCCATG 1608  
ATTTAAAATA TATATTTTAA AATAAATATT TTCTACTATT AAACATAAAAA AAAAAAAAAA 1668  
AAA 1671

644660:964460

Sequence ID No.: 5  
Sequence length: 1437  
Sequence type: Nucleic acid  
Number of strands: Double-strand  
Topology: Straight chain  
Source:

Biological name: Perilla (Perilla frutescens)

Tissue type: Leaf

Direct source:

Library name: cDNA library

Clone name: p8R6

Sequence:

TTCAAACTC	ATAACGTGAT	TGAGCTAATG	TGCACATCTT	CCTCTTCAAA	GTCTACAGTG	60
TCATCCTACC	AGCATCATCA	TGATCAATCT	CTTTATAATG	AGGAGAATGG	AGTAACAAGG	120
AGTGGGTTTT	GTTACTCAGC	TTCAACCTAC	GTACGTACTA	CTACTGACTC	AACTCTCAAG	180
AGAATGAATA	TAATATATAA	TGGGCGATAG	ATCTTTGTAG	ATATGTAGGT	GTAGCCTGCA	240
GGTGGTTAAT	TAATTTCCGG	TGTGGGAAAA	TAAATAAATA	AATAAATATA	GCG ATG AGC	299
					Met Ser	
					1	
AGC AGC AGC AGC AGA AGG TGG AGA GAG AAT GAG GGG ATG CGA AGG ACA						347
Ser Ser Ser Ser Arg Arg Trp Arg Glu Asn Glu Gly Met Arg Arg Thr						
5	10	15				
TTG CTG GGG TTG GGT TTG GGG CAG TTG GTT TCT TTC GAT TTG GCT ATC						395
Leu Leu Gly Leu Gly Leu Gly Gln Leu Val Ser Phe Asp Leu Ala Ile						
20	25	30				
ATG ACC TTT TCT GCT TCT TTG GTT TCA ACC ACA GTG GAT GCA CCA CTT						443
Met Thr Phe Ser Ala Ser Leu Val Ser Thr Thr Val Asp Ala Pro Leu						
35	40	45	50			
ACT ATG TCG TTC ACT ACA TAC ACT GTT GTG GCC CTG CTC TAT GGA ACC						491
Thr Met Ser Phe Thr Thr Tyr Thr Val Val Ala Leu Leu Tyr Gly Thr						
55	60	65				
ATC TTG CTT TAC CGC CGC CAC AAA TTC TTG GTT CCA TGG TAC TGG TAT						539
Ile Leu Leu Tyr Arg Arg His Lys Phe Leu Val Pro Trp Tyr Trp Tyr						
70	75	80				

GCT CTC CTG GGG TTC GTG GAC GTC CAC GGC AAT TAT CTT GTT AAT AAA	587
Ala Leu Leu Gly Phe Val Asp Val His Gly Asn Tyr Leu Val Asn Lys	
85 90 95	
GCA TTC GAG TTG ACA TCG ATT ACG AGT GTG AGC ATA CTG GAT TGT TGG	635
Ala Phe Glu Leu Thr Ser Ile Thr Ser Val Ser Ile Leu Asp Cys Trp	
100 105 110	
ACA ATC GTG TGG TCC ATC ATC TTT ACA TGG ATG TTC CTA GGC ACA AAA	683
Thr Ile Val Trp Ser Ile Ile Phe Thr Trp Met Phe Leu Gly Thr Lys	
115 120 125 130	
TAC TCT GTA TAC CAG TTT GTC GGT GCT GCT ATT TGT GTA GGA GGC CTC	731
Tyr Ser Val Tyr Gln Phe Val Gly Ala Ala Ile Cys Val Gly Gly Leu	
135 140 145	
CTC CTC GTG CTT CTT TCC GAC TCA GGG GTC ACT GCT GCT GGT TCG AAT	779
Leu Leu Val Leu Leu Ser Asp Ser Gly Val Thr Ala Ala Gly Ser Asn	
150 155 160	
CCT CTT TTG GGT GAT TTT CTT GTC ATA ACA GGC TCT ATT TTG TTC ACA	827
Pro Leu Leu Gly Asp Phe Leu Val Ile Thr Gly Ser Ile Leu Phe Thr	
165 170 175	
CTC AGC ACT GTT GGT CAG GAA TAC TGC GTG AAG AGG AAA GAT CGT ATT	875
Leu Ser Thr Val Gly Gln Glu Tyr Cys Val Lys Arg Lys Asp Arg Ile	
180 185 190	
GAA GTA GTA GCA ATG ATC GGT GTA TTT GGT ATG CTC ATC AGT GCA ACC	923
Glu Val Val Ala Met Ile Gly Val Phe Gly Met Leu Ile Ser Ala Thr	
195 200 205 210	
GAG ATT ACT GTG CTG GAG AGG AAT GCC CTC TCA TCA ATG CAG TGG TCT	971
Glu Ile Thr Val Leu Glu Arg Asn Ala Leu Ser Ser Met Gln Trp Ser	
215 220 225	
ACT GGA CTT TTG GCA GCC TAT GTT GTT TAT GCA CTG TCC AGC TTC CTC	1019
Thr Gly Leu Leu Ala Ala Tyr Val Val Tyr Ala Leu Ser Ser Phe Leu	
230 235 240	
TTC TGC ACA CTC ACC CCT TTT CTT CTC AAG ATG AGT GGC GCT GCA TTT	1067
Phe Cys Thr Leu Thr Pro Phe Leu Leu Lys Met Ser Gly Ala Ala Phe	
245 250 255	
TTC AAT CTT TCC ATG CTT ACA TCT GAT ATG TGG GCT GTT GCA ATT AGG	1115
Phe Asn Leu Ser Met Leu Thr Ser Asp Met Trp Ala Val Ala Ile Arg	
260 265 270	

0044965 0044965

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Sequence ID No.: 6
Sequence length: 2105
Sequence type: Nucleic acid
Number of strands: Double-strand
Topology: Straight chain
Source:
    Biological name: Petunia
    Tissue type: Leaf
Direct source:
    Library name: cDNA library
    Clone name: pSPGT1
```

AGTGAGCGCA	ACGCAATTAA	TGTGAGTTAG	CTCACTCATT	AGGCACCCCA	GGCTTTACAC	60
TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG	GATAACAATT	TCACACAGGA	120
AACAGCTATG	ACCATGATTA	CGCCAAGCTC	GAAATTAACC	CTCACTAAAG	GGAACAAAAG	180
CTGGAGCTCC	ACGCGGTGGC	GGCCGCTCTA	GAAGTAGTGG	ATCCCCGGG	CTGCAGGAAT	240
TCCGTTGCTG	TCGCCACAAT	TTACAAACCA	AGAAATTAAG	CATCCCTTTC	CCCCCCTTAA	300
AAAACATACA	AGTTTTTAAT	TTTTCACTAA	GCAAGAAAAT	ATG GTG CAG CCT CAT GTC		358
				Met Val Gln Pro His Val		



ATC	TTA	ACA	ACA	TTT	CCA	GCA	CAA	GGC	CAT	ATT	AAT	CCA	GCA	CTT	CAA	406
Ile	Leu	Thr	Thr	Phe	Pro	Ala	Gln	Gly	His	Ile	Asn	Pro	Ala	Leu	Gln	
			10					15					20			
TTT	GCC	AAG	AAT	CTT	GTC	AAG	ATG	GGC	ATA	GAA	GTG	ACA	TTT	TCT	ACA	454
Phe	Ala	Lys	Asn	Leu	Val	Lys	Met	Gly	Ile	Glu	Val	Thr	Phe	Ser	Thr	
			25					30					35			
AGC	ATT	TAT	GCC	CAA	AGC	CGT	ATG	GAT	GAA	AAA	TCC	ATT	CTT	AAT	GCA	502
Ser	Ile	Tyr	Ala	Gln	Ser	Arg	Met	Asp	Glu	Lys	Ser	Ile	Leu	Asn	Ala	
			40					45					50			
CCA	AAA	GGA	TTG	AAT	TTC	ATT	CCA	TTT	TCC	GAT	GGC	TTT	GAT	GAA	GGT	550
Pro	Lys	Gly	Leu	Asn	Phe	Ile	Pro	Phe	Ser	Asp	Gly	Phe	Asp	Glu	Gly	
55						60					65				70	
TTT	GAT	CAT	TCA	AAA	GAC	CCT	GTA	TTT	TAC	ATG	TCA	CAA	CTT	CGT	AAA	598
Phe	Asp	His	Ser	Lys	Asp	Pro	Val	Phe	Tyr	Met	Ser	Gln	Leu	Arg	Lys	
				75					80					85		
TGT	GGA	AGT	GAA	ACT	GTC	AAA	AAA	ATA	ATT	CTC	ACT	TGC	TCT	GAA	AAT	646
Cys	Gly	Ser	Glu	Thr	Val	Lys	Lys	Ile	Ile	Leu	Thr	Cys	Ser	Glu	Asn	
				90					95					100		
GGA	CAG	CCT	ATA	ACT	TGC	CTA	CTT	TAC	TCC	ATT	TTC	CTT	CCT	TGG	GCA	694
Gly	Gln	Pro	Ile	Thr	Cys	Leu	Leu	Tyr	Ser	Ile	Phe	Leu	Pro	Trp	Ala	
			105					110						115		
GCA	GAG	GTA	GCA	CGT	GAA	GTT	CAC	ATC	CCT	TCT	GCT	CTT	CTT	TGG	AGT	742
Ala	Glu	Val	Ala	Arg	Glu	Val	His	Ile	Pro	Ser	Ala	Leu	Leu	Trp	Ser	
			120					125						130		
CAA	CCA	GCA	ACA	ATA	TTG	GAC	ATA	TAT	TAC	TTC	AAC	TTT	CAT	GGA	TAT	790
Gln	Pro	Ala	Thr	Ile	Leu	Asp	Ile	Tyr	Tyr	Phe	Asn	Phe	His	Gly	Tyr	
135						140					145				150	
GAA	AAA	GCT	ATG	GCT	AAT	GAA	TCC	AAT	GAT	CCA	AAT	TGG	TCC	ATT	CAA	838
Glu	Lys	Ala	Met	Ala	Asn	Glu	Ser	Asn	Asp	Pro	Asn	Trp	Ser	Ile	Gln	
				155					160					165		
CTT	CCC	GGG	CTT	CCA	CTA	CTG	GAA	ACT	CGA	GAT	CTT	CCT	TCA	TTT	TTA	886
Leu	Pro	Gly	Leu	Pro	Leu	Leu	Glu	Thr	Arg	Asp	Leu	Pro	Ser	Phe	Leu	
				170					175					180		
CTT	CCT	TAT	GGT	GCA	AAA	GGG	AGT	CTT	CGA	GTT	GCA	CTT	CCA	CCA	TTC	934
Leu	Pro	Tyr	Gly	Ala	Lys	Gly	Ser	Leu	Arg	Val	Ala	Leu	Pro	Pro	Phe	
			185					190						195		

AAA	GAA	TTG	ATA	GAC	ACA	TTA	GAT	GCT	GAA	ACC	ACT	CCT	AAG	ATT	CTT	982
Lys	Glu	Leu	Ile	Asp	Thr	Leu	Asp	Ala	Glu	Thr	Thr	Pro	Lys	Ile	Leu	
200			205			210										
GTG	AAT	ACA	TTT	GAT	GAA	TTA	GAG	CCT	GAG	GCA	CTC	AAT	GCA	ATT	GAA	1030
Val	Asn	Thr	Phe	Asp	Glu	Leu	Glu	Pro	Glu	Ala	Leu	Asn	Ala	Ile	Glu	
215			220			225			230							
GGT	TAT	AAG	TTT	TAT	GGA	ATT	GGA	CCG	TTG	ATT	CCT	TCT	GCT	TTC	TTG	1078
Gly	Tyr	Lys	Phe	Tyr	Gly	Ile	Gly	Pro	Leu	Ile	Pro	Ser	Ala	Phe	Leu	
235			240			245										
GGT	GGA	AAT	GAC	CCT	TTA	GAT	GCT	TCA	TTT	GGT	GGT	GAT	CTT	TTT	CAA	1126
Gly	Gly	Asn	Asp	Pro	Leu	Asp	Ala	Ser	Phe	Gly	Gly	Asp	Leu	Phe	Gln	
250			255			260										
AAT	TCA	AAT	GAC	TAT	ATG	GAA	TGG	TTA	AAC	TCA	AAG	CCA	AAT	TCA	TCA	1174
Asn	Ser	Asn	Asp	Tyr	Met	Glu	Trp	Leu	Asn	Ser	Lys	Pro	Asn	Ser	Ser	
265			270			275										
GTT	GTT	TAT	ATA	TCT	TTT	GGG	AGT	CTA	ATG	AAT	CCA	TCT	ATT	AGC	CAA	1222
Val	Val	Tyr	Ile	Ser	Phe	Gly	Ser	Leu	Met	Asn	Pro	Ser	Ile	Ser	Gln	
280			285			290										
ATG	GAG	GAG	ATA	TCA	AAA	GGG	TTG	ATA	GAC	ATA	GGA	AGG	CCG	TTT	TTA	1270
Met	Glu	Glu	Ile	Ser	Lys	Gly	Leu	Ile	Asp	Ile	Gly	Arg	Pro	Phe	Leu	
295			300			305			310							
TGG	GTG	ATA	AAA	GAA	AAT	GAA	AAA	GGC	AAA	GAA	GAA	GAG	AAT	AAA	AAG	1318
Trp	Val	Ile	Lys	Glu	Asn	Glu	Lys	Gly	Lys	Glu	Glu	Glu	Asn	Lys	Lys	
315			320			325										
CTT	GGT	TGT	ATT	GAA	GAA	TTG	GAA	AAA	ATA	GGA	AAA	ATA	GTT	CCA	TGG	1366
Leu	Gly	Cys	Ile	Glu	Glu	Leu	Glu	Lys	Ile	Gly	Lys	Ile	Val	Pro	Trp	
330			335			340										
TGT	TCA	CAA	CTT	GAA	GTT	CTA	AAA	CAT	CCA	TCT	TTA	GGA	TGT	TTT	GTT	1414
Cys	Ser	Gln	Leu	Glu	Val	Leu	Lys	His	Pro	Ser	Leu	Gly	Cys	Phe	Val	
345			350			355										
TCT	CAT	TGT	GGA	TGG	AAT	TCA	GCC	TTA	GAG	AGT	TTA	GCT	TGT	GGA	GTG	1462
Ser	His	Cys	Gly	Trp	Asn	Ser	Ala	Leu	Glu	Ser	Leu	Ala	Cys	Gly	Val	
360			365			370										
CCA	GTT	GTG	GCA	TTT	CCT	CAA	TGG	ACA	GAT	CAA	ATG	ACA	AAT	GCC	AAA	1510
Pro	Val	Val	Ala	Phe	Pro	Gln	Trp	Thr	Asp	Gln	Met	Thr	Asn	Ala	Lys	
375			380			385			390							

CAA GTT GAA GAT GTG TGG AAA AGT GGA GTA AGA GTG AGA ATA AAT GAA	1558
Gln Val Glu Asp Val Trp Lys Ser Gly Val Arg Val Arg Ile Asn Glu	
395 400 405	
GAT GGT GTT GTT GAA AGT GAG GAA ATC AAA AGG TGT ATT GAA TTG GTA	1606
Asp Gly Val Val Glu Ser Glu Glu Ile Lys Arg Cys Ile Glu Leu Val	
410 415 420	
ATG GAT GGA GGA GAG AAA GGG GAA GAA TTG AGA AAG AAT GCT AAG AAA	1654
Met Asp Gly Gly Glu Lys Gly Glu Glu Leu Arg Lys Asn Ala Lys Lys	
425 430 435	
TGG AAA GAA TTG GCT AGA GAA GCT GTG AAG GAA GGT GGA TCT TCA CAC	1702
Trp Lys Glu Leu Ala Arg Glu Ala Val Lys Glu Gly Gly Ser Ser His	
440 445 450	
AAG AAT TTA AAG GCT TTT ATT GAT GAT GTT GCC AAA GGG TTT TAATATTTAC	1754
Lys Asn Leu Lys Ala Phe Ile Asp Asp Val Ala Lys Gly Phe	
455 460 465 468	
AGGCTTTTGC CGTGATATTA CTTCCCCTAG TTGGCGATTC ACTCTTTGTG GACTTGCTTG	1814
ACAAAAAACT GAGGGAATGT GCTAAGACAC GCTAATGCTT TAAGAAGTCA TTTCCAAGGC	1874
TTGAAGCCTG CTTTTAAAAC TTATTAGCCA GTAATCTATA GGGTTCTCTT CTATTTTCT	1934
CTGTCTCTCT TTTTAGCCTT TTTCTTTCCA AGGTTTAAGA ATAGCGTGAA CATAGCTTAG	1994
TACGTAGTCT TGGTATCTCT ATCTTACCAA GTGCAAGATT ATGCTTATGC TGTCTCCTA	2054
AATTTCTTAA TAAAATGCAA GATGAAAAAG TACAAAAAAA AAAAAAAAAA A	2105

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## Declaration and Power of Attorney For Patent Application

### 特許出願宣言書及び委任状

### Japanese Language Declaration

### 日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者であると（下記の名称が複数の場合）信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

GENE CODING FOR A PROTEIN HAVING

GLYCOSIDE TRANSFER ACTIVITY

上記発明の明細書（下記の欄でx印がついていない場合は、本書に添付）は、

the specification of which is attached hereto unless the following box is checked:

☐ 月 日に提出され、米国出願番号または特許協定条約国際出願番号を \_\_\_\_\_ とし、  
（該当する場合） \_\_\_\_\_ に訂正されました。

☒ was filed on July 16, 1998  
as United States Application Number or  
PCT International Application Number  
PCT/JP98/03199 and was amended on  
\_\_\_\_\_ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されたとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

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私は、米国法典第35編119条(a)-(d)項又は365条(b)項に基づき下記の、米国以外の国の少なくとも一カ国を指定している特許協力条約365(a)項に基づき国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

### Prior Foreign Application(s)

外国での先行出願 9-200571 (Pat. Appln.)	Japan
(Number) (番号)	(Country) (国名)
(Number) (番号)	(Country) (国名)

私は、第35編米国法典119条(e)項に基づいて下記の米国外特許出願規定に記載された権利をここに主張いたします。

(Application No.) (出願番号)	(Filing Date) (出願日)
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(Application No.) (出願番号)	(Filing Date) (出願日)
-----------------------------	------------------------

(Application No.) (出願番号)	(Filing Date) (出願日)
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私は、私自身の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

### Priority Not Claimed

優先権主張なし

25/July/1997
(Day/Month/Year Filed) (出願年月日)

☐

(Day/Month/Year Filed) (出願年月日)
-----------------------------------

☐

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)
-----------------------------	------------------------

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Status: Patented, Pending, Abandoned) (現況: 特許許可済、係属中、放棄済)
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(Status: Patented, Pending, Abandoned) (現況: 特許許可済、係属中、放棄済)
---

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## Japanese Language Declaration (日本語宣言書)

委任状: 私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。(弁理士、または代理人の氏名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith (list name and registration number)

William L. Mathis	17,337	Robert G. Mukai	28,531	William H. Benz	25,952
Peter H. Smolka	15,913	George A. Hovanec, Jr.	28,223	Peter K. Skiff	31,917
Robert S. Swecker	19,885	James A. LaBarre	28,632	Richard J. McGrath	29,195
Platon N. Mandros	22,124	E. Joseph Gess	28,510	Matthew L. Schneider	32,814
Benton S. Duffett, Jr.	22,030	R. Danny Huntington	27,903	Michael G. Savage	32,596
Joseph R. Magnone	24,239	Eric H. Weisblatt	30,505	Gerald F. Swiss	30,113
Norman H. Stepno	22,716	James W. Peterson	26,057	Michael J. Ure	33,089
Ronald L. Grudziecki	24,970	Teresa Stanek Rea	30,427	Charles F. Wieland III	33,096
Frederick G. Michaud, Jr.	26,003	Robert E. Krebs	25,885	Bruce T. Wieder	33,815
Alan E. Kopecki	25,813	William C. Rowland	30,888	Todd R. Walters	34,040
Regis E. Slutter	26,999	T. Gene Dillahunt	25,423		
Samuel C. Miller, III	27,360	Patrick C. Keane	32,858		
Ralph L. Freeland, Jr.	16,110	Bruce J. Boggs, Jr.	32,344		

書類送付先

Send Correspondence to:  
Ronald L. Grudziecki  
BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404

直接電話連絡先: (名前及び電話番号)

Direct Telephone Calls to: (name and telephone number)

Ronald L. Grudziecki  
at (703) 836-6620

唯一または第一発明者名

Full name of sole or first inventor

Masako Mizutani

発明者の署名

日付

Inventor's signature

Date

*Masako Mizutani* March 12, 1999

住所

Residence

Kyoto-shi, Kyoto, Japan

国籍

Citizenship

Japanese

私書箱

Post Office Address

53-60, Katsurainui-cho, Nishikyo-ku,

Kyoto-shi, Kyoto, Japan

第二共同発明者

Full name of second joint inventor, if any

Yoshikazu Tanaka

第二共同発明者

日付

Second inventor's signature

Date

*Yoshikazu Tanaka* March 12, 1999

住所

Residence

Otsu-shi, Shiga, Japan

国籍

Citizenship

Japanese

私書箱

Post Office Address

2-7-4, Oginosato, Otsu-shi, Shiga, Japan

(第三以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for third and subsequent joint inventors.)

第三共同発明者	Full name of third joint inventor, if any <u>Takaaki Kusumi</u>	
第三共同発明者	日付	Third inventor's signature <u>Takaaki Kusumi</u> Date March 12, 1999
住 所	Residence Suita-shi, <u>Osaka</u> , Japan	
国 籍	Citizenship Japanese	
私書箱	Post Office Address 2-12-21-402, Yamate-cho, Suita-shi, Osaka,	
	Japan	
第四共同発明者	Full name of fourth joint inventor, if any <u>Kazuki Saito</u>	
第四共同発明者	日付	Fourth inventor's signature <u>Kazuki Saito</u> Date March 12, 1999
住 所	Residence Yachimata-shi, <u>Chiba</u> , Japan	
国 籍	Citizenship Japanese	
私書箱	Post Office Address 663-86, Ekido, Yachimata-shi, Chiba, Japan	

第五共同発明者	Full name of fifth joint inventor, if any <u>Mami Yamazaki</u>	
第五共同発明者	日付	Fifth inventor's signature <u>Mami Yamazaki</u> Date March 12, 1999
住 所	Residence Chiba-shi, <u>Chiba</u> , Japan	
国 籍	Citizenship Japanese	
私書箱	Post Office Address 4-12-6, Benten, Chuo-ku, Chiba-shi, Chiba,	
	Japan	
第六共同発明者	Full name of sixth joint inventor, if any <u>Gong Zhizhong</u>	
第六共同発明者	日付	Sixth inventor's signature <u>Gong Zhizhong</u> Date March 12, 1999
住 所	Residence Chiba-shi, <u>Chiba</u> , Japan	
国 籍	Citizenship Chinese	
私書箱	Post Office Address 201, Namikikopo, 12-5, Inagehigashi 3-chome,	
	Inage-ku, Chiba-shi, Chiba, Japan	

(第七以降の共同発明者についても同様に記載し、署名をすること)

(Supply similar information and signature for seventh and subsequent joint inventors.)